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Effect of Nitrogen and Phosphorus Enrichment on Lipid and Fatty Acid Composition of Estuarine Microplankton

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EFFECT OF NITROGEN AND PHOSPHORUS ENRICHMENT ON LIPID AND FATTY ACID COMPOSITION OF ESTUARINE MICROPLANKTON

A Thesis

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Of the Requirements for the Degree of

Master of Arts

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by

Jia Chen

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APPROVAL SHEET

This thesis is submitted in partial fulfillment

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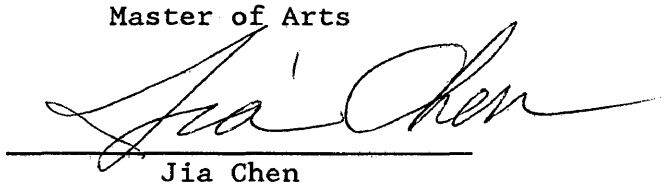

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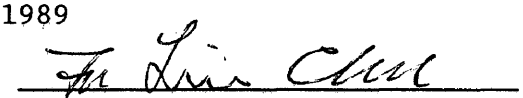

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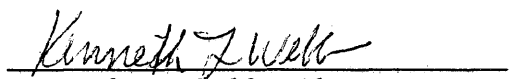

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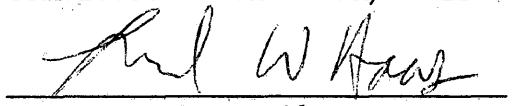

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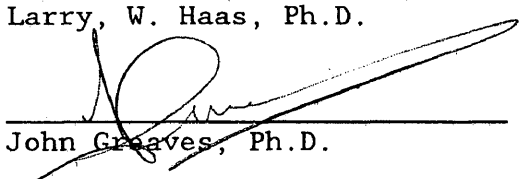

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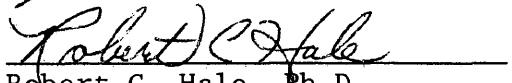

Robert C. Hale, Ph.D.

DEDICATION

This thesis is dedicated to my mother and brothers for their love and encouragement, and also to the memory of my father, who has always been an example for me.

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ABSTRACT

The purpose of this study is to investigate the effects of nutrient enrichment on the lipid and fatty acid composition of the microplankton community of the York River estuary, Virginia, U.S.A. The bioassay experiments employed 50 L outdoor continuous cultures inoculated with natural microplankton (<60um) from the York River estuary. Responses of the microplankton community to nutrient enrichment were dependent upon the time of the year, the magnitude of nutrient supplements, and the dilution rate. In general, microplankton food quality in the York River estuary cannot always be improved by either nutrient limitation or nutrient enrichment, because nutrient limitation can cause low biomass, while nutrient enrichment results in a deficiency of lipids and essential fatty acids in the cells. Dilution rate was shown to be important in regulating biomass, intracellular lipid storage and essential fatty acid concentrations of the microplankton community. Results of this study suggest that management strategies to improve the Chesapeake Bay ecosystem should consider the time of the year as well as the magnitude of the nutrient input.

EFFECT OF NITROGEN AND PHOSPHORUS ENRICHMENT ON LIPID AND FATTY ACID COMPOSITION OF ESTUARINE MICROPLANKTON

INTRODUCTION

Lipids and fatty acids of microplankton have been extensively studied in recent years because of their important biological functions. These compounds are considered to be the most important source of energy to the marine food web. Microplankton (<60 um), both phototrophic and heterotrophic, are considered to be the major contributors of lipids and fatty acids. Unicellular algae serve as primary producers and as potential food sources for microzooplankton, which in turn serve as food sources for higher level marine organisms (Anonymous, 1966). Lipids in microplankton also serve as an energy reservoir, buoyancy controls and as thermal insulators (Gagosian and Lee, 1981).

Microplankton release dissolved lipids and fatty acids into sea water. This release may have a subtle ecological effect (Kattner et al., 1983; Parrish, 1988) due to its inhibition of growth in other species of microplankton (Procter, 1957; Gauthier and Aubert, 1981). The toxicity of fatty acids to marine organisms may be attributed to their surface active properties, or it may be the result of formation of toxic oxidation products (Hashimito, 1979). Release of lipids and fatty acids may also be a means of complexing trace metals (Mantoura, 1981).

Lipids, particularly fatty acids, can be used as biomarkers of marine organisms, because these compounds are easily extracted,

identified and quantified. Biomarkers can define not only the distribution of microplankton, but also their physiological status at different growth stages and under different environmental conditions (Sargent et al., 1988; Currie and Johns, 1988). Much work has been done to study the effects of environmental factors on the lipid metabolism of microplankton. Polh and Zurheide (1979) have shown that algae grown under high light intensities tend to have higher concentrations of omega-3 polyunsaturated fatty acids (PUFA) in their total lipids, while other studies (Ackman et al., 1968; Seto et al., 1984) have shown that decreasing temperature has a similar effect.

Despite extensive study on lipid and fatty acid metabolism of microplankton, many problems still remain unsolved. The current work was initiated by curiosity about some very basic, but crucial questions.

With the exception of a few reports (Morris and Carre, 1984; Dikarev, 1987; Morris, 1984), all investigations of lipid and fatty acid metabolism of microplankton have been conducted on cultured species. Such data, however useful, are not necessarily directly related to natural food chains, since significant differences in fatty acid composition between cultured samples and field samples have been observed (Morris and Culkin, 1976; Ballantine et al., 1979). This leads to the question: **what controls the lipid and fatty acid metabolism of natural estuarine microplankton?**

The food quality of microplankton depends not only on biomass, but also on lipids stored in the cells, a concept neglected by many

researchers. In addition, omega-3 (w3) and omega-6 (w6) fatty acids, especially ecosapentaenoic (20:5w3) and docosahexaenoic (22:6w3) acids, are essential for many marine animals (Yu and Sinnhuber, 1972; Kanazawa et al., 1977; Langdon and Waldock, 1981; Webb and Chu, 1983). Deficiency of these fatty acids in microplankton can result in high mortality of marine animals who feed on these microorganisms.

Lipid and fatty acid characteristics of microplankton are highly dependent on culture conditions (Ackman et al., 1964; Pugh, 1971; Tornabene et al., 1974). Under optimum growth conditions, algae contain an abundance of protein and low content of lipids. The reduced levels of lipids, which are a source of energy, reduce the effectiveness of algae as a food to marine animals (Anonymous, 1966). It has been proposed that regulating culture conditions might be helpful to overcome the deficiency of lipids. There is considerable evidence in cultured species that fat accumulation takes place in response to the exhaustion of the nitrogen supply in the medium. As the nitrogen depletion progresses, protein content decreases and carbohydrate content increases. This is followed by an increase in lipid content (Milner, 1948; Spoehr and Milner, 1949; Myers, 1951; Miller, 1962; Richardson et al., 1969).

Quantitative and qualitative variations of natural enclosed microplankton lipids at different growth stages have been studied by Morris et al. (1985). Their work showed increases of both carbohydrates and lipids occurred towards the end of a bloom as nitrate and silicate in the water were depleted. However,

information on the response of lipid metabolism of natural estuarine microplankton to different nutrient treatments is still scarce. Question: **Is the food value of natural living estuarine microplankton affected by variations of environmental nutrient conditions?**

The response of microplankton communities to nutrient enrichment in the coastal ecosystem is of major significance in marine ecology and has broad implications in terms of both water quality and fisheries. Chesapeake Bay is the largest and most productive estuary in the U.S. Recent work has suggested that in the York River, a tributary of Chesapeake Bay, microplankton growth is naturally nitrogen limited in summer and phosphorus limited in winter (Webb, 1988). However, any nonpoint (e.g. runoff and ground water) or point (e.g. sewage discharge) nutrient input may initiate the transition of such limitation (D'Elia, 1985). Enough symptoms of overenrichment of nutrients in Chesapeake Bay (Heinle et al., 1980; Brush 1984; Officer et al., 1984; Price et al., 1985) have been shown to warrant concern about regulation of nutrient input. There has been a bay-wide controversy over the importance of nitrogen and phosphorus enrichment on the nutrient limitation of algae (D'Elia, 1985). One major concern is that increased biomass production resulting from nutrient enrichment may enhance the anoxia or hypoxia in areas such as the central Chesapeake Bay (Officer et al., 1984). Some other possible effects of nutrient enrichment may have been neglected. One of these is the variation of lipid and fatty acid content of microplankton communities which may severely

affect the marine food web. Question: **What is the seasonal response of lipid and fatty acid content of natural estuarine microplankton communities to nutrient enrichment?**

To answer the questions posed above, an outdoor 50 L continuous culture technique was selected. The reasons to choose such an approach in this study are similar to D'Elia et al. (1986). First, cell densities in the culture are maintained close to natural levels, so that light limitation is less likely. Secondly, continuous culture can be sustained long enough to recognize the species succession effect. Finally, continuous culture is amenable to studies of community structure (Tilman et al., 1982). Since the York River estuary exhibits strong seasonal variations in freshwater flow, dilution rates in the continuous culture were manipulated to mimic such effects. In addition, the concentrations of added nutrients (both nitrogen and phosphorus) were also manipulated to investigate the response of the microplankton community to nutrient enrichments of different magnitudes.

In summary, the purposes of this study were to determine: (1) the lipid and fatty acid content of natural estuarine microplankton under different nutrient treatments over a seasonal cycle; (2) to what extent microplankton under artificial nutrient enrichment differ from those under nutrient controlled and natural conditions.

MATERIALS AND METHODS

Study Site

The experiments were conducted on a research pier at the Virginia Institute of Marine Science (VIMS), Gloucester Point, Virginia, between July 1988 and March 1989. The pier is located in the lower York River estuary of the lower Chesapeake Bay (Fig. 1).

Microplankton Culture

A small scale continuous flow biological culture system was used in this study. This technique has been successfully used at VIMS for the nutrient studies in microplankton communities (Webb, 1986), and the experimental results have shown good consistancy and replication.

Six experiments were conducted between July 1988 and March 1989. The culture conditions are summarized in Table 1. For the October-March experiments, each consisted of three nutrient treatments (two additions and a control). Each of the nutrient treatments were conducted at two dilution rates, low and high. Thus there were six treatments (3 nutrient treatments X 2 dilution rates) per experiment. The experiment conducted in July, 1988 was a preliminary experiment, in which only one dilution rate was used and N:P ratios of added nutrients were different from other experiments.

Prior to the experiments, all the fiberglass culture tanks (37cm diameter, 50 cm high) were bleach washed and then rinsed at least

three times with fresh water. The nutrient tanks were sterilized with 95% ethanol and all the tubing was autoclaved. Natural microplankton from the York River estuary were freshly inoculated by pumping the size fractioned (<60 um) York River water (YRW) into the 50 L microcosms. The culture tanks were continuously diluted with 0.2 um filtered YRW, using peristaltic pumps, at rates of 0.25 and 0.5*culture volume*day^{-1} (d^{-1}). Nutrient medium was prepared by dissolving NH_4Cl and NaH_2PO_4 in 0.2 um filtered YRW at defined N:P molar ratios (e.g. 5:1 and 16:1, Table 1). An initial addition of both nitrogen and phosphate was made at the beginning to bring nutrient concentrations to the required input levels. The microcosms were exposed to natural sunlight screened to 50% incidence, and ambient temperature was maintained by circulating YRW around the microcosms. Mixing was provided to each microcosm, by aeration, at a rate of approximately 1.8 L per minute through 15 cm air stones.

Sampling

Cultures from each microcosm were analyzed daily for temperature, salinity and chlorophyll-a (chl-a). When the steady growth stage was reached, according to the chlorophyll-a readings (6-10 days), samples were collected from each microcosm for lipid and nutrient (dissolved and particulate) analyses. Microplankton for lipid analysis were sampled by continuous centrifugation of the entire 50 L microcosm culture through 0.6 um porosity filters.

The filters were then stored under nitrogen in 20 ml chloroform-methanol (2:1 v/v) at -70°C until analyzed. For each experiment, 50 L of ambient York River water were also collected, using the same method.

Species Analysis

Information on cell composition and biovolume were provided by Eldridge (1989), using an epifluorescence microscopy technique (Haas, 1982). Biovolume was obtained by measuring the plane of randomly selected cells to yield the volume of an equivalent solid, using standard geometric shapes.

Chemical Analysis

Chlorophyll-a was sampled by filtering 5 ml of the microcosm culture through 47 mm GF/F filters, and then extracting with 8 ml dimethylsulfoxide -acetone-distilled water (DH_2O) (45:45:10 v/v) plus 0.1% diethylamine for at least 24 hrs. The analysis of extracts was carried out daily with a Turner Designs Fluorometer. Duplicate 50 ml aliquots of sample were filtered through 25 mm GF/F filters and stored frozen until particulate organic carbon (POC) and nitrogen (PON) analyses which were performed on a Perkin-Elmer Model 240B CHN Elemental Analyzer. Particulate organic phosphorus (POP) was hydrolyzed and analyzed as phosphate with an Orion AutoAnalyzer. Samples for dissolved nutrient (phosphate, ammonia, nitrate and nitrite) analyses were filtered through 47 mm GF/F filters and

frozen for later analysis on a Technicon AutoAnalyzer (D'Elia et al., 1983).

Lipid and Fatty Acid Analysis

Total lipids were extracted from wet microplankton with chloroform-methanol-DH_2O (1:2:0.8 v/v) according to the procedure of Bligh and Dyer (1959). Total lipid in each sample was measured gravimetrically.

Half of each total lipid sample was used for lipid composition analysis. Lipid classes were separated into a polar lipid fraction and the other nine neutral lipid classes by thin layer chromatography (TLC) using precoated silica gel plates (Applied Science Laboratories, State College, PA) and a developing solvent of hexane-ethyl ether-acetic acid (85:15:1 v/v). Quantification of each lipid fraction was accomplished by a charting-densitometry technique (Chapelle, 1977) using a GS-300 Transmittance/Reflectance scanning densitometer (Hoefer Scientific Instruments, San Francisco, CA) and expressed as a percentage of the total lipids.

In order to determine the fatty acid composition, the other half of the total lipid sample was methylated with methanol and boron-trifluoride (BF_3) (Morrison and Smith, 1964). Selection of this procedure was based on comparison with two other transmethylation methods (Chen and Chu, unpublished data). The methanol-BF_3 method used in this study provided good reproducibilities and recovery of the fatty acids on algal samples

and required only small amounts of solvent. Impurities were removed from the fatty acid methyl esters (FAME) by TLC, using precoated silica gel plates with absorption bands (Applied Science Laboratories, State College, PA) and a developing solvent of hexane-ethyl ether-acetic acid (90:10:1 v/v). Separation of the FAME was carried out by gas liquid chromatography (GLC, Varian 3700 and 3300). The chromatographs were equipped with flame ionization detectors, and used polar (OV-351) and nonpolar (DB-5) fused silica capillary columns (30m X 0.32mm i.d., J & W Scientific Inc., Folsam, CA). The columns were temperaure-programmed from 70-220^{o}C at 7^{o}C/min for the polar column and 140-320^{o}C at 6^{o}C/min for the nonpolar column; the flow rates of compressed air and hydrogen to the detector were 300ml/min and 30ml/min, respectively; helium was used as the carrier gas at 3.0ml/min; splitless injection mode was used for these analyses.

Identification of FAME was based on comparison of retention time with commercial standards and with a secondary reference standard (FAME of cod liver oil) (Ackman and Burgher, 1965). Identifications were further confirmed with an Extrel 400-2 GC-MS system using chemical ionization. Areas of FAME peaks were integrated with a computer system (Chromatochart-PC, Interactive Microware Inc., PA) connected to the GLC. Results were expressed as a percentage of total fatty acids. The results were corrected with response factors based on the comparison of peak areas between

samples and commercial quantitative fatty acid standards, and the recovery of each fatty acid (Chen and Chu, unpublished data).

Statistical Analysis

The effects of time (seasonal variation), nutrient treatment and dilution rate (high and low) on the lipid content of natural estuarine microplankton were examined. Each of these three factors impacted the lipid metabolism in the natural microplankton community, singly or in combination with the other(s). Multiple analysis of variance (MANOVA), and where appropriate ANOVA, were performed on the data. P values were reported. When P was less than 0.05, the parameter was considered to have made a significant contribution to the variance.

RESULTS

NUTRIENT AVAILABILITY AND BIOLOGICAL PARAMETERS OF YRW

Environmental conditions of YRW during the experimental period are listed in Table 2. In the York River estuary, water temperature and light intensity were positively related throughout the experimental period. The exception was in the March, when light intensity started to increase, while water temperature remained low. Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) both increased disproportionately from July through October, resulting in an increase in the N:P ratio from 2.21 to 12.6. This was followed by the annual switch from N limitation to P limitation (N:P>16) as DIP became depleted, and the N:P ratio increased to 19.7. During December and January, both DIN and DIP started to decrease, again disproportionately, resulting in a further increase in the N:P ratio. In March, inorganic nitrogen in YRW started to rise. Inorganic phosphorus, however, decreased further, and the N:P ratio reached 120.

Seasonal variations of chlorophyll-a and lipid content of ambient York River microplankton are summarized in Table 3. Chlorophyll-a decreased between July and December, followed by a 7-fold increase in January, and then a decrease in March. Minor changes in the total lipid content in YRW were observed (1.28 fold increase) between July and mid-October, while lipid content per unit chlorophyll-a increased more than three fold (26.3 to 89.5 ug/ug

chl-a). Total lipid content per unit chlorophyll-a declined from mid-October till January, and then increased in March.

SPECIES COMPOSITION

Species composition of the microplankton community in the microcosms is summarized in Table 4. The results demonstrated the existence of multispecies communities in the microcosms. Quantitatively, diatoms were the dominant species in the community in most experiments. According to Fig. 2, diatoms dominated all treatments in the microcoms in July and October (>92.9% of the biovolume). When winter approached (December and January), diatoms became less predominant. In December, heterotrophic bacteria became abundant (av. 14.9% of the biovolume). In January, phototrophic nanoflagellates were dominant, dependant upon the treatments (e.g. 93.3% of biovolume in the treatment of N:P 5:1 with high dilution rate). This induced the large variations in the diatom as percentage of total biovolume. Overall, phototrophic nanoflagellates comprised 25.5% (av.) of the biovolume in January. When spring came (March), diatoms resumed their dominance (av. 85.8% of the biovolume).

BIOMASS

Chlorophyll-a was used as a biomass index of the total microplankton, including both heterotrophic and phototrophic in this study. Chlorophyll-a is not always an appropriate biomass index of microplankton, since the chlorophyll:carbon ratio of phytoplankton

varies with light intensity. In addition, chlorophyll-a represents only phototrophic microplankton. The relationships between POC and chlorophyll-a were analyzed (Fig. 3). Chlorophyll-a and POC were significantly related ($y=40.4x+0.46$, $R^2=0.671$, $P=0.000$). As a result, community biomass can be adequately represented by chlorophyll-a content.

The seasonal distribution of community biomass of the microcosm microplankton is demonstrated in Fig. 4. In the summer (July), biomass was moderate in the microcosm (av. 18.2 ug/L). When fall approached (mid-October), an increase of over five fold in biomass (av. 99.2 ug/L) was observed, after which biomass started to decline.

The enrichment response of the microplankton community was evaluated by means of a response ratio, which is a comparison of certain biological parameters of the nutrient-enriched microcosms to the nutrient-controlled microcosms. A ratio of 1.0 indicates no response to enrichment; a ratio larger than one indicates the parameter was enhanced due to enrichment; and a ratio less than one indicates the parameter was decreased due to enrichment.

Fig. 5 demonstrated the biomass response to the nutrient enrichment. Positive response ratios occurred in July (av. 2.71) mid-October (av. 3.69), January (av. 8.86) and March (av. 30.3). During the remainder of the year, no significant response was observed. Between July and January, response ratios within the same microcosm experiment appeared to be very close regardless of

differences in either the N:P ratio or dilution rate. In March, however, the response ratios varied between 19.8 and 66.8, dependant on the culture conditions. During this period, addition of nutrients with high N:P ratio (16:1) enhanced the community biomass more intensively than the nutrients with low N:P ratio (5:1) (ANOVA, P=0.01). Meanwhile, for a given N:P ratio, higher biomass was yielded with the high dilution rate than with the low dilution rate (ANOVA, P=0.02). Since only one dilution rate was used in the July experiment, the effect of dilution rate cannot be discussed.

TOTAL LIPIDS

Total community lipid content showed similar temporal trends with community biomass (compare Fig. 4 and 6). Significant response of the total lipid content occurred in mid-October, January and March, similar to biomass. However, response ratios of the community total lipid content differed with respect to biomass (Fig. 7). Response ratios of total lipid content were on a much smaller scale (response ratio of 0.95 to 4.38), compared to the biomass (0.41 to 66.8).

In addition to the biomass and total lipid content, which were indicators of community behavior, lipid storage in the cells, expressed by lipid content per unit biomass, was calculated by dividing total community lipid content by community biomass (ug lipids/ug chl-a). Fig. 8 contains the data of lipid content per unit biomass from each microcosms. This parameter appeared to be very different from biomass and total lipid content. In mid-October

when biomass was the highest, lipid content per unit biomass was the lowest. There was another interesting phenomenon: in the spring, an enormous lipid accumulation took place in the controls. The lipid content per unit biomass reached 153 ug/ug chl-a. Response ratios of intracellular lipid storage to nutrient enrichment are illustrated in Fig. 9. As indicated above, the intracellular lipid storage in the controls were generally higher than the nutrient-enriched microcosms (response ratio<1). It has therefore been shown that nutrient enrichment tended to deplete the lipid storage in the cell (response ratio less than 1.0) at most times of the year, except late-October (response ratio of 1.82). In July, mid-October and January, lipid storage in the cell was depleted by 50% due to addition of nutrients. In March, however, there was about 90% was diminition of the lipid storage as a result of nutrient addition.

A regression analysis of the relationship between intracellular nutrients and lipid content was performed. Both total community lipid content and lipid content per unit biomass were related with PON and POP (Fig. 10). When the nutrients were taken up and accumulated in the intracellular pool, enhancement of community total lipids occurred. Lipid content per unit biomass, on the other hand, tended to decrease with increased PON and POP. However, when the intracellular N:P ratio was considered, it showed no relationship to the total community lipid content (MANOVA, P=0.774) and the lipid content per unit biomass (MANOVA, P=0.190).

LIPID COMPOSITION

Microplankton lipids were classified into two basic classes: polar lipids (PL) and neutral lipids (NL). NL were further separated into nine categories: monoglycerides (MG); diglycerides (DG); alcohols (ALC); sterols (ST); free fatty acids (FFA); triglycerides (TG); fatty acid esters (FAE). sterol esters (SE); and wax esters (WE). PL plus NL equalled 100% of the total lipids.

Ambient Microplankton Lipid composition of ambient microplankton are summarized in Appendix I. Triglycerides, which are the major energy containing component in NL, showed fairly consistent levels during the experimental period with a mean of 21.6% of total lipids. Natural York River estuarine microplankton contained large amounts of MG (mean of 10.2%) and small amounts of both DG (mean of 1.9%) and ALC (mean of 3.0%). The averages of ST and SE content were 6.1% and 4.3%, respectively. No FAE were found in any ambient microplankton samples. Free fatty esters and wax esters, two very important NL, were both present in the natural microplankton. In ambient York River microplankton, FFA, which were fairly constant over the year comprised 4.6% of total lipids. Wax esters, 6.3% of total lipids, have rarely been found in algae and bacteria. Wax esters in marine copepods (Sargent et al., 1976; Lee and Nevenzel, 1979) and the phytyl esters in marine dinoflagellates (Withers and Nevenzel, 1977) were considered to be the only two possible contributors to the WE pools in microorganisms, according to published papers.

Microcosm Microplankton Unlike the ambient microplankton, lipid composition of microplankton in the microcosms demonstrated strong variations, dependent on both season of year and culture conditions (Appendix I). Triglycerides increased nearly 100% by addition of nutrients in the summer (from av. 21.9% to 42.6%). During the fall season, nutrient enrichment had little effect on the TG content. When the winter approached (December and January), triglycerides were depleted by addition of nutrients (from av. 26.9% to 14.6%). The microplankton in the controls at this stage tended to conserve TG in such nutrient-depleted medium. In the spring, triglycerides showed only a very minor response to the addition of N and P. During the fall season (October), FFA content in nutrient-enriched treatments tended to show a 3-7 fold increase over the summer season. In the spring, little response of FFA to the addition of nutrients was shown. Wax esters in this study varied from 2.1% to 15.8% of the total lipids. This component has rarely been found in bacteria and algae. Sterol esters varied from 2.3% to 16.2% of the total lipids. Lee et al. (1971) also found that ST accounted for as much as 17% of the lipids in marine diatoms. Alcohols, monoglycerides and diglycerides constituted from <0.5% to 4.8%, 1.3% to 12.9% and <0.5% to 18.8% of the total lipids, respectively. Polar lipids, structural components of cell membranes, appeared to be less abundant in the nutrient-enriched microcosms (av. 30.3%) than in natural estuarine microplankton (av. 42.1%).

Triglycerides function as an energy reservoir. Polar lipids, on the other hand, function as menbrane structural components, which

limit the microplankton production. Low PL can inhibit microplankton growth. The relationships between these two components, having very different biological roles, were analyzed (Fig. 11). These two lipid fractions were negatively related.

FATTY ACID COMPOSITION

Ambient Microplankton The fatty acid composition of natural York River microplankton during the experimental period is summarized in Appendix II. Among the saturated fatty acids, which comprised 40.4 to 65.5% of the total fatty acids, 16:0 was the predominant one and comprised 24.6 to 43.8%. 16:1 and 18:1 (both w7 and w9) were the only monoenoic fatty acids. They constituted 10.4 to 14.0% and 2.7 to 9.2% of the total fatty acids, respectively. Polyunsaturated fatty acids (PUFA), which have important biological and physiological functions, varied between 15.9 and 37.3% of the total fatty acids. The linolenic type (w3) and linoleic type (w6) fatty acids dominated the PUFA (>88%). Interestingly, omega-3 and omega-6 fatty acids showed almost opposite trends during the experimental period (Fig. 12). Omega-3 fatty acids in the fall and winter (av. 21.9%) were at least two times more abundant than in the summer and spring (av. 6.03%). Omega-6 fatty acids, however, were more abundant in summer and spring (av. 10.5%) than in fall and winter (av. 3.11%), except late-October. Between July and mid-October, 18:1 declined, accompanied by enhancement of 18:3w3 and 18:4w3, which then declined. There were increases of 20:5w3 and 22:6w3

between mid- and late-October. In the spring, both 20:5w3 and 22:6w3 were extremely depleted and PUFA with 18 carbons prevailed.

Long chain hydrocarbons having molecular masses between 308 and 462, at intervals of 14 were present in the ambient microplankton samples at high concentrations (Appendix II). The tentative identifications of these compounds based on mass spectra were monounsaturated alkenes, having the general formula of C_nH_{2n}, where n equals integers between 22 and 33. The amount of these long chain hydrocarbons exceeded fatty acids over the year cycle (ALK/FA>1), except for the period of December and January.

Microcosm microplankton The fatty acid composition of microplankton in the microcosms are listed in Appendix III. Fatty acid composition varied with season, dilution rate and nutrient concentration. 14:0 and 16:0 were the most abundant among the saturated fatty acids which comprised 29.8 to 66.9% of the total lipids, and their predominance oscillated. In the summer and winter, concentrations of 16:0 were obviously higher than 14:0 (ANOVA, P=0.001). The rest times of the year, 14:0 and 16:0 were similar. Comparing the two monoenoic fatty acids, 16:1 and 18:1 (both w7 and w9), 16:1 was more abundant in the nutrient-enriched microplankton community. In the controls, 18:1 was enhanced and became predominant in March. There was another peculiar phenomenon observed, only <0.05% of w3 fatty acids were detected in the controls in March. The only PUFA quantified in March was 18:2w6. Throughout the year, w3 fatty acids generally exceeded w6 (w3/w6>1)

(ANOVA, P=0.03), except in July when w6 prevailed (w3/w6<1) (ANOVA, P=0.05). In the summer (July), both 20:5w3 and 22:6w3 were minor constituents of PUFA. Unexpectedly 20:4w6, which was <0.05% in the other seasons, represented 4.9% (av.) of the total fatty acids in the summer. Consequently, concentrations of w6 fatty acids were enhanced by this unexpected fatty acid. Based on the results in Appendix III, the concentrations of 22:5w3 and 22:6w3 varied between <0.05% to 15.6% (av. 8.8%) and <0.05% to 6.5% (av. 2.7%) of the total lipids, respectively. Relationships between PUFA and intracellular nutrients were analyzed. Total PUFA, w3 fatty acids and 20:5w3 demonstrated relationships with intracellular N:P ratios (Fig. 13). All these fatty acids appeared to decline with increased N:P ratio.

The same group of long chain hydrocarbons found in ambient York River microplankton was also present in the microcosm samples. Ratios of ALK/FA were not affected by different nutrient treatments (MANOVA, P=0.100), time (MANOVA, P=0.164) and dilution rate (ANOVA, P=0.483). The ratio varied between 0.09 and 3.7, and the highest occurred in the nutrient-controlled community in March.

DISCUSSION

SEASONAL VARIATIONS

The three major factors contributing to the seasonal variations of microplankton biomass and lipid content in YRW are temperature, light intensity and nutrient availability. The last one will be discussed separately. It has been reported that increased temperatures tend to promote algal growth and result in higher biomass, but meanwhile, lipid and fatty acid content in the cells is reduced (Kleinschmidt and McMahon, 1970a). Increased light intensity, however, tends to decrease both chlorophyll and lipid content in the cells (Constantopoulos and Bloch, 1967). Such effects of temperature and light intensity contributed to the strong seasonal fluctuations of biomass (MANOVA, $P=0.00$), community total lipid content (MANOVA, $P=0.00$) and lipid content per unit biomass (MANOVA, $P=0.05$) in YRW and microcosms.

Polar lipids, which are the structural components of cell membranes, reflect biosynthetic pathways and membrane structural requirements (Carroll, 1965; Wolfe et al., 1965; Jezky and Penicnak, 1966), and are therefore closely related to microplankton production. Characteristically, the photochemical apparatus of photosynthetic organisms is rich in PL containing mono- and digalactose diglycerides, which are regulated by both light intensity and temperature. In this study, polar lipids were more abundant in the season with low light and low temperature (December

and January). The two PL components (mono- and digalactose diglycerides) were inversely related with light intensity in this study. Such phenomenon has also been observed by Constantopoulos and Bloch (1967). On the other hand, membranes of the microplankton contain more PL component, particularly glycolipids and phospholipids, which play important roles in thermostability. Variation of such PL components is an indication of temperature fluctuation. Increasing PL content with decreasing temperature is in agreement with the results of Kleinschmidt and McMahon (1970b). They interpreted this phenomenon as lower solubility of carbon dioxide at higher temperature, forcing the normally autotrophic cells to grow more like heterotrophic cells which contain somewhat degenerated chloroplasts and thus contain less glycolipids and phospholipids.

Polyunsaturated fatty acids of the microplankton in microcosms showed strong seasonal variations (MANOVA, $P=0.02$). Since most of the PUFA (16:3, 16:4, 18:2 and 18:3 etc.) are typical constituents of the chloroplasts, light plays a key role in regulating these components. Enhancement of PUFA by increasing light intensity has been observed in different organisms by various researchers (Nichols, 1965; Constantopoulos and Bloch, 1967). In contrast, content of PUFA in this study was low in July (av. 13.8% of total lipids) when light intensity was high. Afterwards, content of PUFA increased when light intensity was decreasing between October and January (av. 33.6%). When light intensity started to increase in the spring (March), their levels declined (av. 26.6%). In this

study, the synthesis of PUFA appeared to be more sensitive to temperature than to light. It has been well established that microorganisms living at lower environmental temperatures contain lipids with lower melting points than organisms inhabiting higher environmental temperature regimes (Farrell and Rose, 1967). Thus decreasing temperature is expected to enhance the unsaturation of the fatty acids which reduces the melting point. Low temperature was thought to cause an increase in the PUFA fraction, especially with C_{18} fatty acids (Akman et al., 1968). Results of this study agreed with the hypothesis that low temperature favors PUFA synthesis. Polyunsaturated fatty acids were more abundant in the cold (December and January with av. 36.6% of total fatty acids) than in the hot (July with av. 13.8%) seasons.

NUTRIENT AVAILABILITY

Effects of nutrient enrichment on phytoplankton biomass has been studied by many scientists. The response of the natural microplankton population to the addition of nutrients provides an useful means of determining the factors limiting microplankton growth (Goldman, 1972). The response of phytoplanktonic growth to nutrient enrichment in a tributary of Chesapeake Bay has been studied by D'Elia et al. (1986). In their bioassay experiments, great enhancement of growth by nutrient addition occurred during both the low-flow, late summer season (N limitation with N:P<5:1) and the high-flow, late winter season (P limitation with N:P>90:1).

Significant responses of microplankton biomass in this study, however, occurred in mid-October, January and March. Little response of biomass (response ratio of 1.39) occurred in July when nutrient level was low and N:P ratio was approximately 3 in YRW.

Total community lipid content and lipid content per unit biomass were shown to be closely related to PON and POP, but not with N:P in the cells (Appendix IV). This indicated that concentrations of intracellular nutrients, not N:P ratio, regulated the microplankton production and lipid synthesis. An inverse relationship between lipid content per unit biomass and intracellular nutrients (Fig. 10) indicated that lipid storage in the cell tended to diminish with nutrient addition.

Lipid classes differ in the proportions of O, N and P incorporated in their structures, thus it is reasonable to assume that different nutrient regimes would favor the synthesis of different lipid classes. There are very few studies on the effect of nutrients on intracellular lipid classes. Parrish and Wangersky (1987) suggested that the yield of TG is triggered by N stress, so that TG is more abundant at lower N levels. In this study, TG failed to show any correlation with PON concentration (R^2=0.005, P=0.892). However, there is a strong negative correlation between TG and PL (Fig. 12). Similar results have also been reported by Parrish and Wangersky (1987). It has been suggested that phospholipid and TG share common precursors (Gurr and James, 1980) which are likely to be channeled toward synthesis of one component

if the synthesis of the other component is hindered. This explanation has a very important implication: triglycerides, an energy reserve, and polar lipids, which are regulators of microplankton production, cannot be favored at the same time in their biosynthetic pathways. When microplankton production is promoted, energy storage in the cell diminished.

Nutrient treatments played important roles in regulating PUFA, w3 fatty acids, 20:5w3 and hydrocarbon synthesis (Appendix IV). Nitrogen enrichment is thought to favor the formation of PUFA (Pohl and Zurheide, 1979; Ben-Amotz and Tornabene, 1985). In the current study, the PUFA levels were found to decline with increased N:P ratio (Fig. 13), but they were not related to PON (R^2=0.002, P=0.515) and POP (R^2=0.014, P=0.532). Omega-3 PUFA and 20:5w3 also declined with increased N:P ratio (Fig. 13). These results indicated that nitrogen enrichment or phosphorus limitation, which enhanced the N:P ratio, could seriously damage the PUFA pool in the microplankton. The food quality of the microplankton was reduced.

It is apparent that nutrient enrichment not only stimulates the production of microplankton as predicted, but also changes the food quality of microplankton and consequently affects the whole marine food web.

DILUTION RATE

The theory of a close relationship between dilution rate and growth rate in a continuous culture has been well established. No

obvious relationship between dilution rate and growth, however, was shown in this study, due to interspecific interactions such as grazing and competition in a multispecies microplankton community. Effect of dilution rate on lipid metabolism, however, has been investigated in the current experiments for the first time.

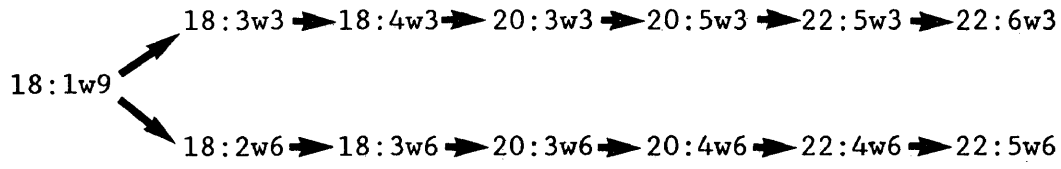
Over a large time scale (yearly), dilution rate had no effect on the lipid content per unit biomass in the microcosms (MANOVA, $P=0.31$), but it did appear to affect the lipid storage in December (ANOVA, $P=0.00$), when little biomass response occurred (Fig 6 and 8).

Microplankton growth rate theoretically varied with dilution rate. In a microplankton community, fatty acid composition would also be expected to respond to such changes. This is because fatty acids tend to regulate heterotrophic microplankton production due to their important nutritional and structural functions. Even though the relationships between most of the fatty acids and the dilution rate were not systematic, the differences in the fatty acid (especially essential fatty acid) yields between the high and low dilution rate cannot be ignored. For example, omega-3 fatty acid concentration was shown to be dependent on dilution rate (MANOVA, $P=0.04$). Between late-October and March, omega-3 content was generally higher with low dilution rate than with high dilution rate (ANOVA, $P=0.02$). As a result, it is reasonable to infer that any changes of dilution rate in YRW, which can be caused by wind, run-off and tidal mixing, can consequently and seriously affect the food quality of the microplankton.

Variations of intracellular composition and biochemical activities of microplankton are likely to occur in a continuous culture, due to the theoretically close relationship between dilution rate and microplankton growth rate. Variations in macromolecular composition and metabolites of microorganisms with dilution rate have been studied by various researchers (e.g. Brown and Stanley, 1972; Cooney et al., 1976). The present results are the first on the effect of dilution rate on the lipid and fatty acid content. Few quantitative conclusions concerning dilution effect can be drawn, since many factors, such as grazing and competition, are difficult to manipulate in a natural microplankton community. Therefore, it would be desirable to investigate the qualitative and quantitative effect of dilution on the lipid metabolism of monospecies microplankton, and then extend this to a natural community.

SYNTHETIC PATHWAYS OF FATTY ACIDS

Each fatty acid is not only affected by environmental and nutrient conditions, as discussed before, but also channeled with other fatty acids along biosynthetic pathways. Akman et al. (1964) postulated the possible biosynthetic pathways shown below:



The current study provided some evidence for this hypothesis. For the ambient York River microplankton (Appendix II), 18:1

concentration was strongly related to the PUFA. Between July and mid-October, 18:1 declined, accompanied by enhancement of 18:3w3 and 18:4w3, which then declined along with increases in 20:5w3 and 22:6w3. This is a good demonstration of the biosynthetic pathways above from precursor (18:1w9) to successor fatty acids (18:3w3, 18:4w3, 20:5w3 and 22:6w3).

It was thought that the enzyme system in the biosynthetic pathways preferred the w3 family to the w6 family (Tinoco et al., 1979). But in this study, the enzyme system could favor either one. For ambient York River microplankton, the omega-3 family was preferred (w3/w6>1) in mid-October, December and January. In July and March, the omega-6 family was preferred (w3/w6<1). In late-October, however, no preference was observed (w3/w6=1). The microplankton in microcosms, the enzyme system favored the w6 family in July, and 20:4w6 was also fairly abundant. During the rest of the year, elongation and desaturation of 18:2w6 to 20:4w6 were severely hindered as in YRW. In the controls in March, the w3 biosynthetic pathway was inhibited, and 18:2w6 was the only PUFA quantified. Phosphorus deficiency might be the cause of this "blocking". "Blocking" is a general phenomenon in mature cells (Ackman et al., 1964). It is suggested by the current study that it also related to nutrient availability in the environment.

ORIGINS OF SOME LIPIDS AND FATTY ACIDS

The relatively large quantities of WE present in this study were interesting. Copepods have traditionally been thought to be

the only marine microorganism containing WE, but copepods were presumed absent from the microcosms in this study. Withers and Nevenzel (1977) later demonstrated the existence of phytyl esters (5% of the total lipids) in marine dinoflagellates which are the only marine algae that reportedly contain such compounds. Wax ester content in this study varied from 2.10 to 15.8% of the total lipids. Apparently, contributors other than dinoflagellates were involved. It seems that WE content was related to the population of phototrophic flagellates, qualitatively, but not quantitatively. Size fraction work needs to be done to confirm this hypothsis. Low levels of ALC (<5%) were found in the experiments. It reflected the ability of bacteria to convert the ALC to the corresponding acids (Gilbertson et al., 1981; Gillan et al., 1983).

Concentrations of 20:5w3 and 22:6w3 attracted most of the attention in this study, because they are essential to marine animals. Both of these PUFA were present in this study and varied from <0.05% to 15.6% and <0.05% to 6.49% of the total fatty acids, respectively. According to previous reports, summarized by Pohl and Zurheide (1979), diatoms, a dominant species in the microcosms, have shown a tremendous amount of 20:5w3 (>50%) and low content of 22:6w3 (<2.2%) in their fatty acid pool. Meanwhile, dinoflagellates, which were also present in the microcosms, have demonstrated an unusual abundance of 22:6w3 (up to 30%) in their fatty acid pool. It is believed that dinoflagellates contributed 22:6w3 to the fatty acid pool in this study. Concentrations of both fatty acids appeared to be lower than in the unispecies microplankton. This effect might be

produced by the coexistence of the diatoms and dinoflagellates. The results demonstrated one difference between multispecies and unispecies communities.

EFFECTS OF NUTRIENT ENRICHMENT ON THE FOOD QUALITY OF YORK RIVER ESTUARINE MICROPLANKTON ON A SEASONAL BASIS

As a major food source for higher trophic animals, the nutritional value of microplankton is as important as its quantity. Lipid is a major energy reserve. Additionally, certain amounts of essential fatty acids (20:5w3 and 22:6w3) are required for animal growth. Biomass, lipids and fatty acids of natural microplankton in the York River demonstrated seasonal variation. The results of this study showed that the addition of N and P disturbed the natural pattern of the microplankton community. As a result, the food quality of microplankton in an estuary could be radically changed. Seasonal change and nutrient variation in the ambient YRW were related. Both DIN and DIP showed significant seasonal patterns. As a result, the extent of the nutrient enrichment effect depended on both time of year and magnitude of the nutrient input. Effects of nutrient enrichment on the food quality are listed in Table 5. From the table, it is seen that even if the nutrients were enriched in the culture media fairly close in time (e.g. Oct. 21 and Oct. 28), the results could be very different. Enrichment on Oct. 21 severely depleted the lipid storage in the microplankton (16.0 to 7.51 ug/ug chl-a) and the food quality was decreased. Nutrient enrichment on Oct. 28, however, improved food quality of microplankton by

increasing lipid content in the cell (13.1 to 23.7 ug/ug chl-a) as well as the content of essential fatty acids (10.4 to 15.2% of the total fatty acids). In the low biomass season (January), when the nutrient concentrations were extremely dilute in YRW, the addition of nutrients helped to improve the food quality by enhancing the microplankton biomass (2.68 to 23.7 ug chl-a/L). In the spring when biomass and essential fatty acids were extreamly depleted, food quality was very poor. By adding nutrients to the water at this time, biomass bloomed, while enormous amounts of 20:5w3 and 22:6w3 accumulated in the cell. Even though there was a significant decrease in the lipid storage of the cells, moderate amounts of lipids remained. Food quality was improved.

Results of this study indicate that the prevailing hypothesis that "food value of microplankton can be improved either by nutrient enrichment to yield high biomass or nutrient limitation to yield high lipid content" needs to be reevaluated. In a natural estuarine microplankton community, nutrient enrichment could enhance the biomass of the microplankton, but would also cause the deficiency of lipid storage in the cells. In addition, enrichment of nitrogen severely reduces the pools of essential fatty acids, which could result in death of aquatic animals. Nutrient limitation, on the other hand, induced the deficiency of food, which could also threaten the animal survival. Obviously, optimal nutritional value of food in a natural estuary is only obtained when all three parameters (biomass, lipids and fatty acids) are optimized.

CONCLUSIONS

This is the first study dealing with the effect of nutrient enrichment on the lipid metabolism of cultured multispecies microplankton communities obtained from a natural estuary. The response of the natural microplankton community to nutrient enrichment is much more complicated than in a mono-species community. The response varies not only with seasonal changes, but also with species composition, community production and physiological status of each cell. For example, nutrient enrichment can induce the predominance of marine diatoms in the York River estuary, and as a result, increase the community biomass. Meanwhile, lipid storage in the cell become severely depleted. Nutrient availability regulates the lipid and fatty acid metabolism of microplankton. In the cells with high intracellular N:P ratios, the internal pool of 20:5w3 and 22:6w3 can be seriously depleted. According to the current study, the conclusion can be drawn that food value of the natural estuarine microplankton cannot always be improved by either nutrient limitation or nutrient enrichment, since they can cause either low community biomass or a deficiency of lipids and essential fatty acids in the cells.

This study is also the first to deal with the effect of dilution rate on the lipid metabolism of natural estuarine microplankton. The role of dilution rate is more than a simple regulation of the community growth rate. Dilution rate, along with

nutrient availability, are factors which control the physiology of the estuarine microplankton community. Dilution rate affects the community biomass, intracellular lipid storage and concentrations of essential fatty acids. This study revealed the possibility of using dilution rate to improve the food quality in a continuous culture of natural microplankton. This apparently cannot be accomplished by either nutrient enrichment or limitation alone.

This study demonstrated the importance of regulating nutrient input in reference to time of year. Such input may initiate a transition of nutrient limitation, which may change the food web.

The study illustrated the complexity of the relationship between a multispecies microplankton community and environmental conditions. Grazing and competition among the species are difficult to manipulate in such a community. For better understanding of natural microplankton communities, size fraction work needs to be done. For example, by analyzing the lipid and fatty acid composition of microplankton in each fraction, we may obtain a clearer picture of how microplankton of different sizes adjust themselves to a given environment, which may in turn seriously affect the animals that feed on such microplankton.

Table 1. Summary of Culture Conditions of Microcosm Experiments

Experiment	Sampling Date	Duration (d)	Salinity ppt	Temp °C
1*	07/21/88	10	20.0	27.1
2	10/21/88	6	23.3	15.2
3	10/28/88	6	23.8	15.0
4	12/09/88	10	21.5	10.0
5	01/13/89	9	20.0	11.8
6	03/30/89	8	18.8	9.3

Supply Ratios of Added Nutrients	umolar N	umolar P
control*	0	0
N : P = 25 : 0*	25	0
N : P = 20 : 1*	50	2.5
N : P = 5 : 1*	25	5.0
N : P = 16 : 1	40	2.5

Dilution Rates		Flow Rates (Ld^{-1})
$0.33d^{-1}$*		16.7
$0.25d^{-1}$	(low)	12.5
$0.50d^{-1}$	(high)	25.0

* Culture conditions in the preliminary experiment.

Table 2

Environmental Conditions in YRW During the Experimental Period

Date	Temp(°C)	Light	DIN(umol)	DIP(umol)	N:P(molar)
07/21/88	27.1	1.26	--	--	--
07/29/88	27.0	1.26	0.38	0.16	2.21
08/05/88	28.9	1.32	2.43	0.65	3.76
08/11/88	29.5	1.32	3.07	0.90	3.40
10/21/88	15.2	0.96	10.1	0.84	12.0
10/28/88	15.0	0.96	13.0	1.03	12.5
12/08/88	10.0	0.77	10.8	0.55	19.7
01/13/89	11.8	0.66	2.01	0.09	22.1
03/30/89	9.3	0.78	3.07	0.03	120.

-- Data lost

a: Monthly averages of daily maximum solar radiation (Langleys/min). Data from VIMS data base.

Table 3

Biomass, Total Lipid Content (TL) and Lipid Content per Unit Biomass (TL/Biomass) of Ambient York River Microplankton During Experimental Period

Date	Biomass (ug chl-a/L)	TL (ug/L)	TL/Biomass (ug/ug chl-a)
07/21/88	13.1 ± 0.0[a]	345 ± 63	26.3 ± 0.5
10/21/88	5.32 ± 1.45	444 ± 93	89.5 ± 4.2
10/28/88	4.39 ± 0.13	264 ± 34	60.1 ± 5.9
12/09/88	3.35 ± 0.70	156 ± 8	48.0 ± 12.5
01/13/89	20.5 ± 0.7	327 ± 72	16.1 ± 4.0
03/30/89	9.62 ± 0.01	306 ± 34	31.8 ± 3.5

a: Standard deviation from triplicates.

Table 4. Microplankton Composition of Microcosm Experiments

AUTOTROPHS		
Procaryotes	**Diatoms**	**Other groups**
cyanobacteria	Ditylum brightwellii	Pyramimonas
	Thalassiosira sp.	Chrysophytes
	Leptocylindrous danica	Haptophytes
	Chaetoceros	Dinoflagellates
	Skeletonema costatum	Cryptophytes
	Stephanopyxis sp.	Micromonas sp.
	Thalassiothrix sp.	
	Nitzschia closteriun	

HETEROTROPHS	
Procaryotes	**Eucaryotes**
bacteria	flagellates
	dinoflagellates
	ciliates

Table 5

Effect of Nutrient Enrichment on the Food Quality of

York River Estuarine Microplankton

	BIOMASS (ug chl-a/L)		TL/BIOMASS (ug/ug chl-a)		ESSENTIAL FA (%)		FOOD QUALITY	
Experiment	Control	Enrichment	Control	Enrichment	Control	Enrichment	Control	Enrichment
07/21/88	7.97	21.6	75.6	38.9	3.20	3.59	POOR	POOR
10/21/88	40.7	128.	16.0	7.51	9.33	17.3	FAIR	POOR
10/28/88	32.9	28.9	13.1	23.7	10.4	15.2	FAIR	GOOD
12/09/88	15.4	15.6	22.9	21.3	12.0	12.3	GOOD	GOOD
01/13/88	2.68	23.7	43.2	18.8	12.9	16.3	FAIR	GOOD
03/30/89	1.39	52.9	172.	18.6	<0.05	12.0	POOR	GOOD

Figure 1. Study site of microcosm experiments in the lower York River estuary.

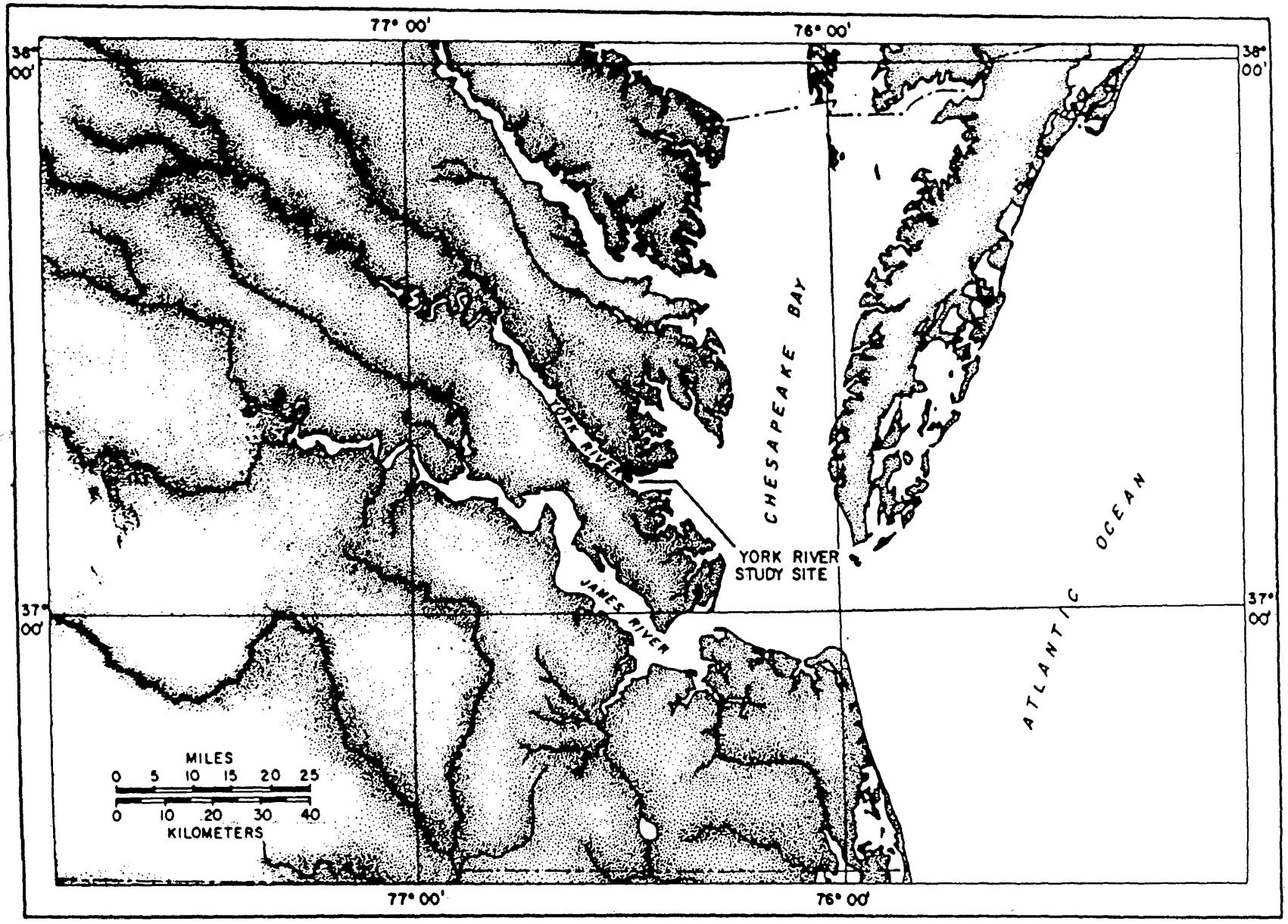

77° 00'
76° 00'
38° 00'
37° 00'
CHESAPEAKE BAY
YORK RIVER
JAMES RIVER
YORK RIVER STUDY SITE
ATLANTIC OCEAN
MILES
0 5 10 15 20 25
0 10 20 30 40
KILOMETERS

Figure 2. Seasonal variations of diatom concentration (as percentage of total microplankton biovolume), each data point represents means of all treatments for that date ($\bar{X} \pm SD$).

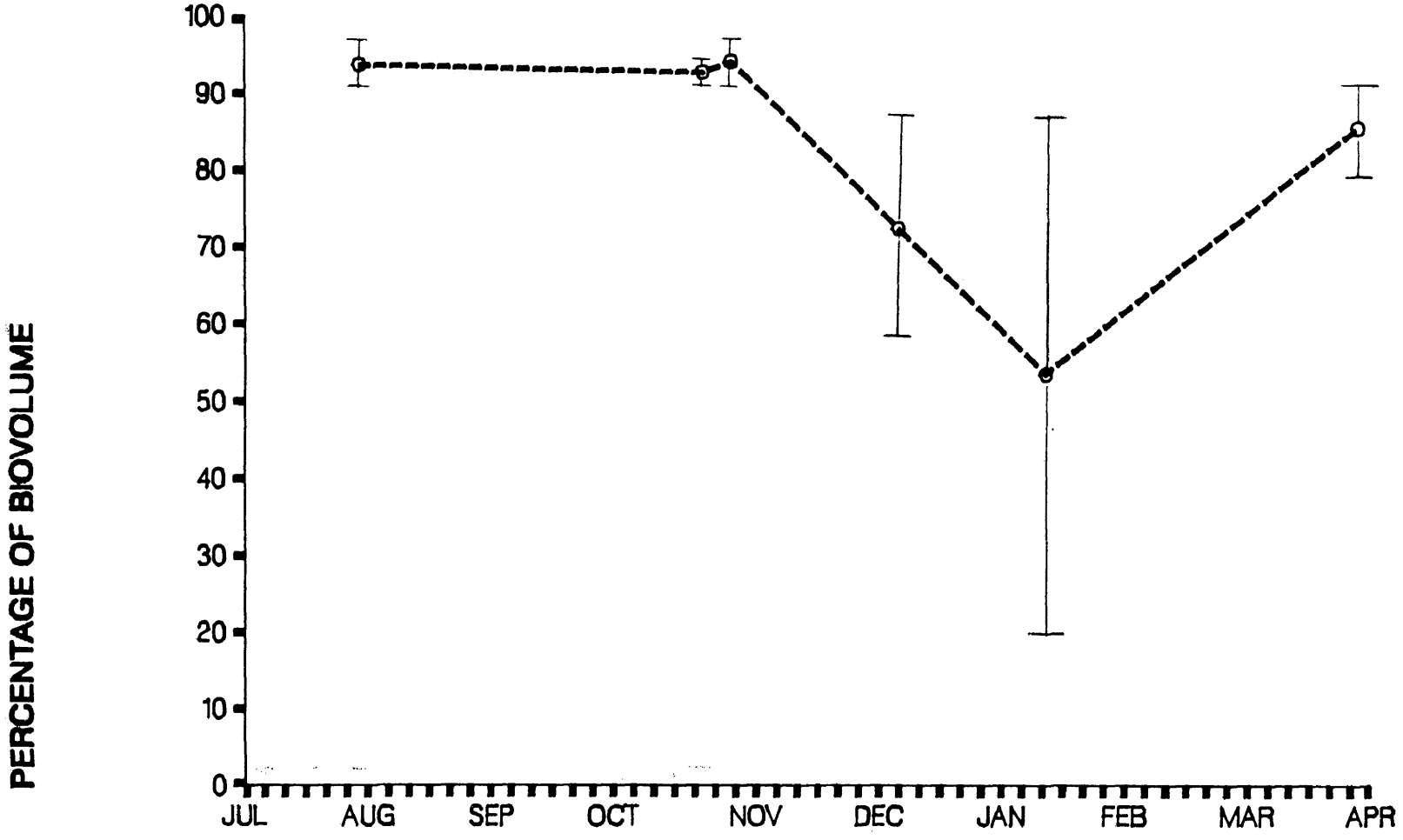

PERCENTAGE OF BIOVOLUME
100
90
80
70
60
50
40
30
20
10
0
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR

Figure 3. POC (mg/L) versus chlorophyll-a content (mg/L).

$y = 40.4x + 0.46$ ($r^2 = 0.671$, $n = 30$, $P = 0.000$)

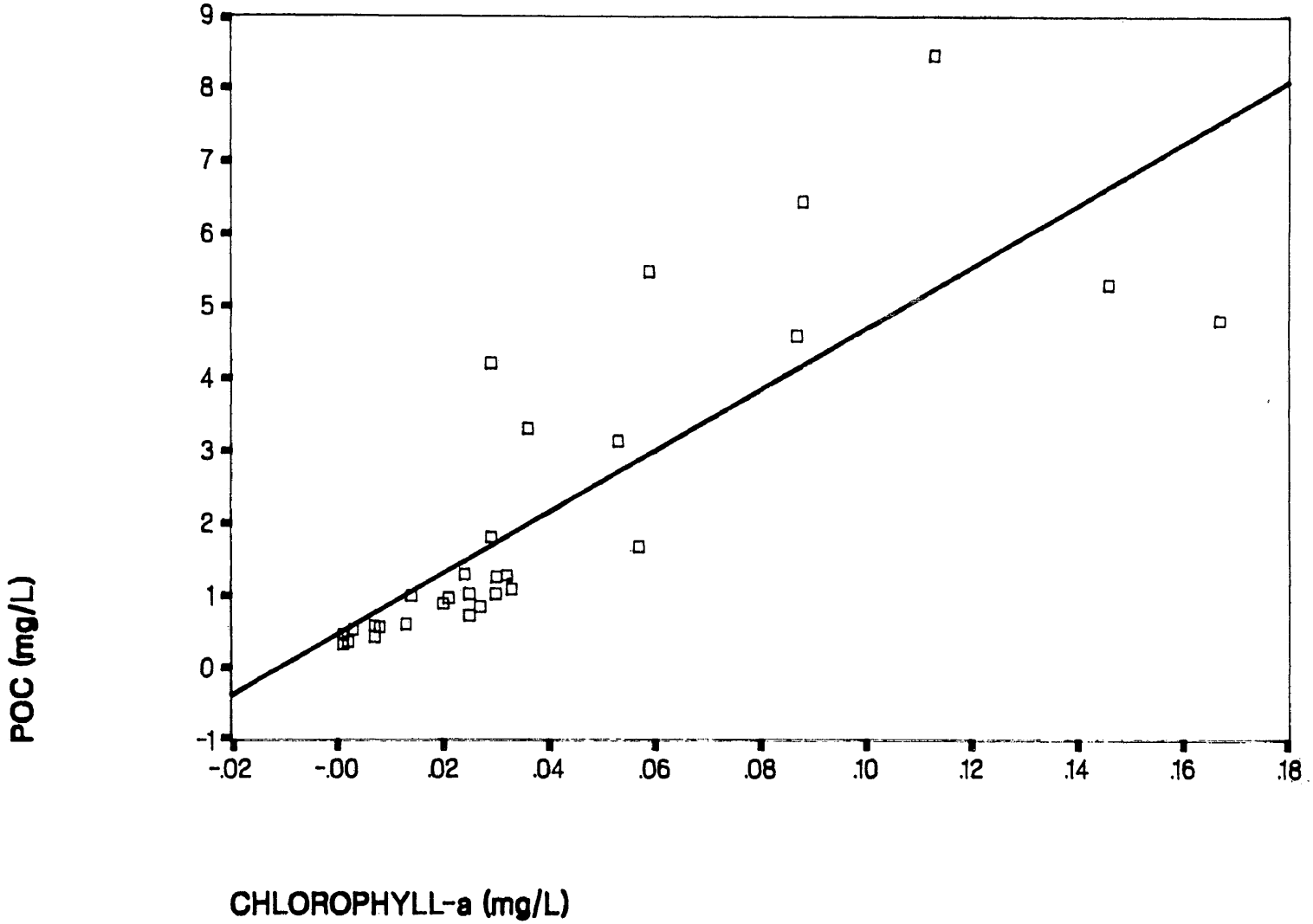

9
8
7
6
5
4
3
2
1
0
-1
-.02
-.00
.02
.04
.06
.08
.10
.12
.14
.16
.18
POC (mg/L)
CHLOROPHYLL-a (mg/L)

Figure 4. Microplankton biomass as chlorophyll-a (ug/L) in the microcosms. Unlined bars: low dilution rate ($0.25d^{-1}$); Solid bars: high dilution rate ($0.5d^{-1}$); Cross-lined bars: dilution rate of $0.33d^{-1}$.

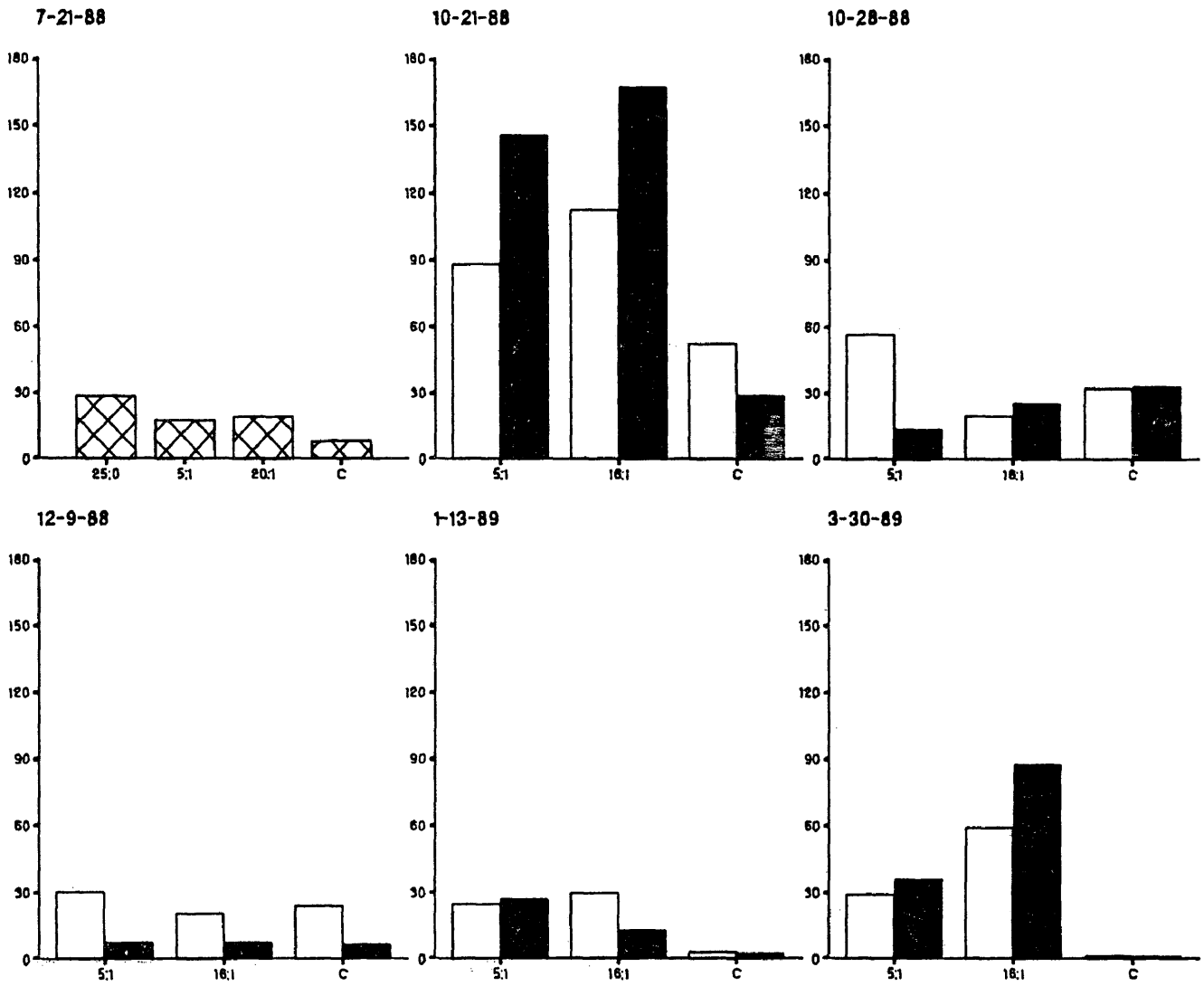

7-21-88
10-21-88
10-28-88
12-9-88
1-13-89
3-30-89
180
150
120
90
60
30
0
25:0
5:1
20:1
C
5:1
16:1
C

Figure 5. Enrichment response ratio of microplankton community biomass in the microcosms. Data in July represented the dilution rate of $0.33d^{-1}$.

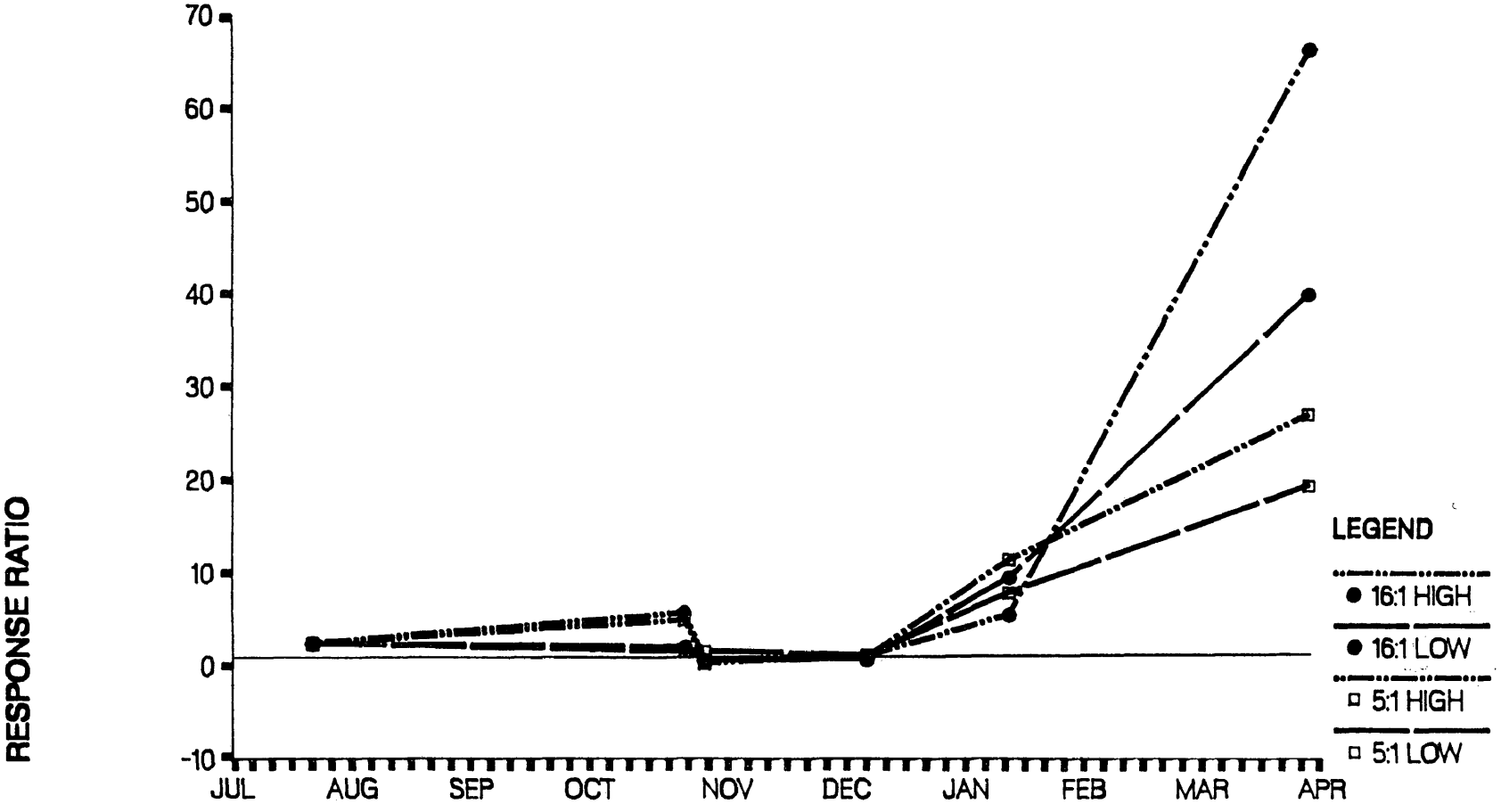

RESPONSE RATIO
70
60
50
40
30
20
10
0
-10
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
LEGEND
16:1 HIGH
16:1 LOW
5:1 HIGH
5:1 LOW

Figure 6. Total lipid content of the microplankton community (mg/microcosm) in the microcosms. Unlined bars: low dilution rate ($0.25d^{-1}$); Solid bars: high dilution rate ($0.5d^{-1}$); Cross-lined bars: dilution rate of $0.33d^{-1}$.

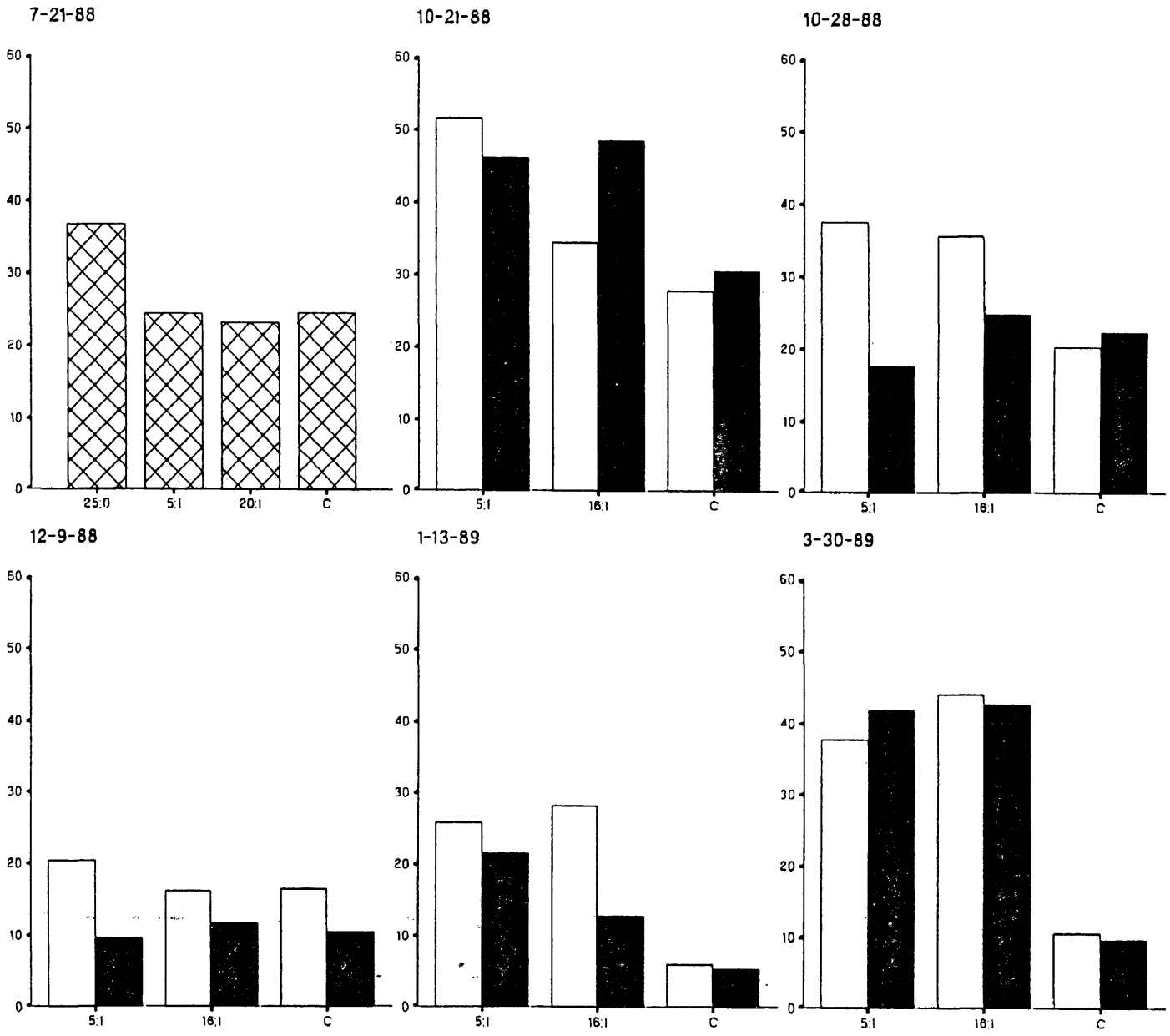

7-21-88
10-21-88
10-28-88
12-9-88
1-13-89
3-30-89
60
50
40
30
20
10
0
25:0
5:1
20:1
C
16:1

Figure 7. Enrichment response ratio of total community lipid content in the microcosms. Data in July represented the dilution rate of $0.33d^{-1}$.

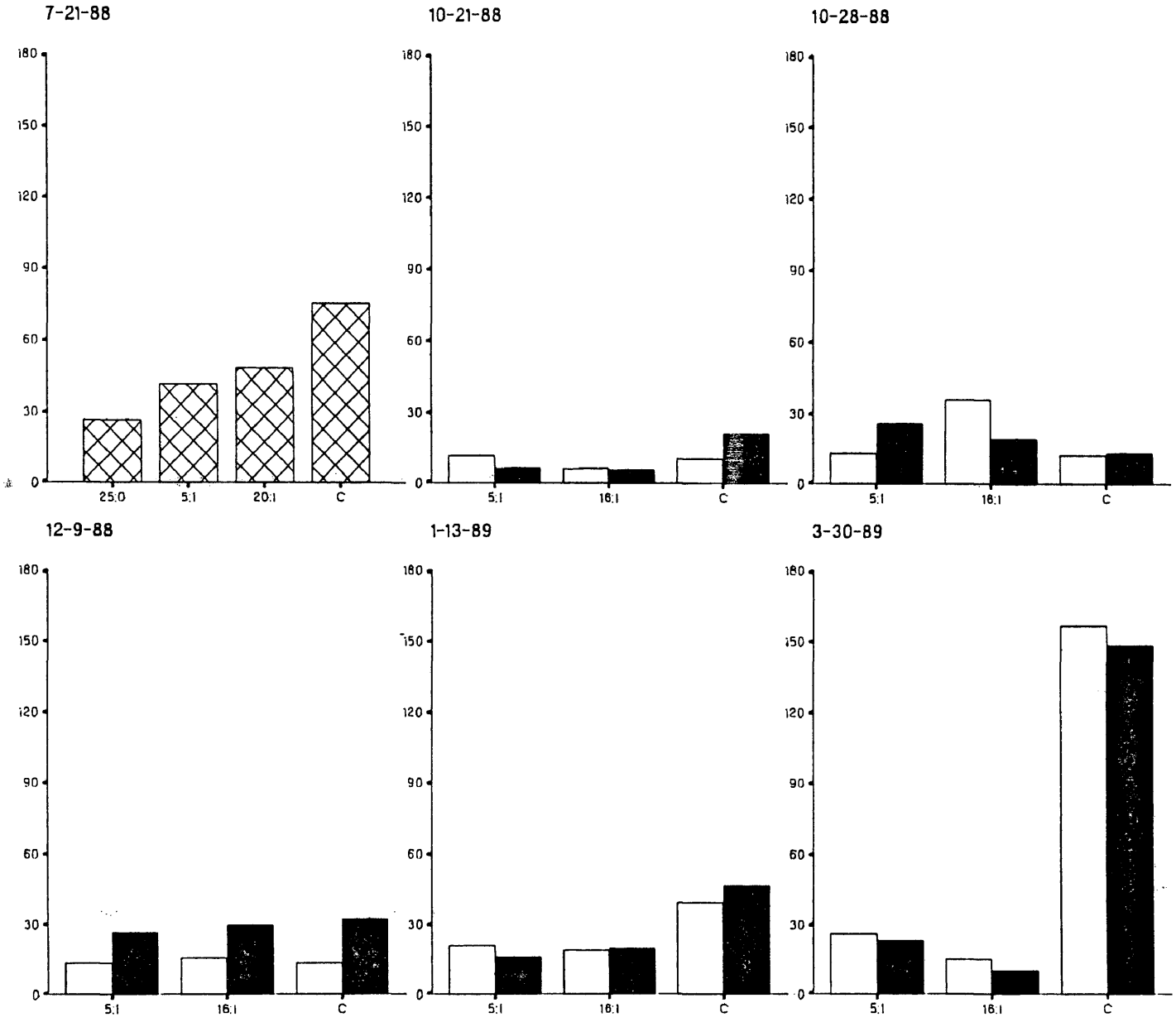

7-21-88
10-21-88
10-28-88
12-9-88
1-13-89
3-30-89
180
150
120
90
60
30
0
25:0
5:1
20:1
C
16:1

Figure 8. Lipid content per unit biomass (ug/ug chl-a) in the microcosms. Unlined bars: low dilution rate ($0.25d^{-1}$); Solid bars: high dilution rate ($0.5d^{-1}$); Cross-lined bars: dilution rate of $0.33d^{-1}$.

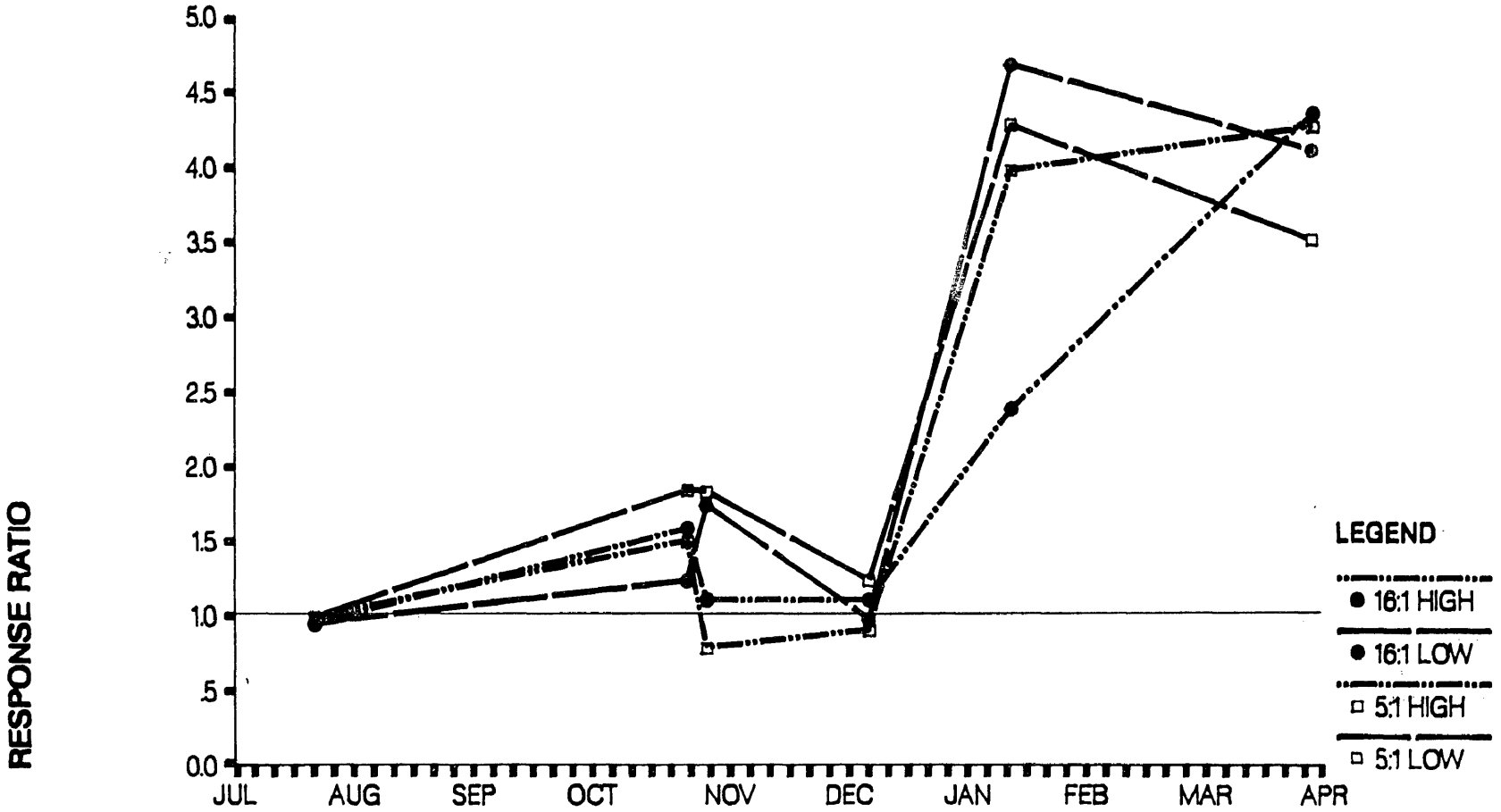

RESPONSE RATIO
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
LEGEND
16:1 HIGH
16:1 LOW
5:1 HIGH
5:1 LOW

Figure 9. Enrichment response ratio of lipid content per unit biomass in the microcosms. Data in July represented the dilution rate of $0.33d^{-1}$.

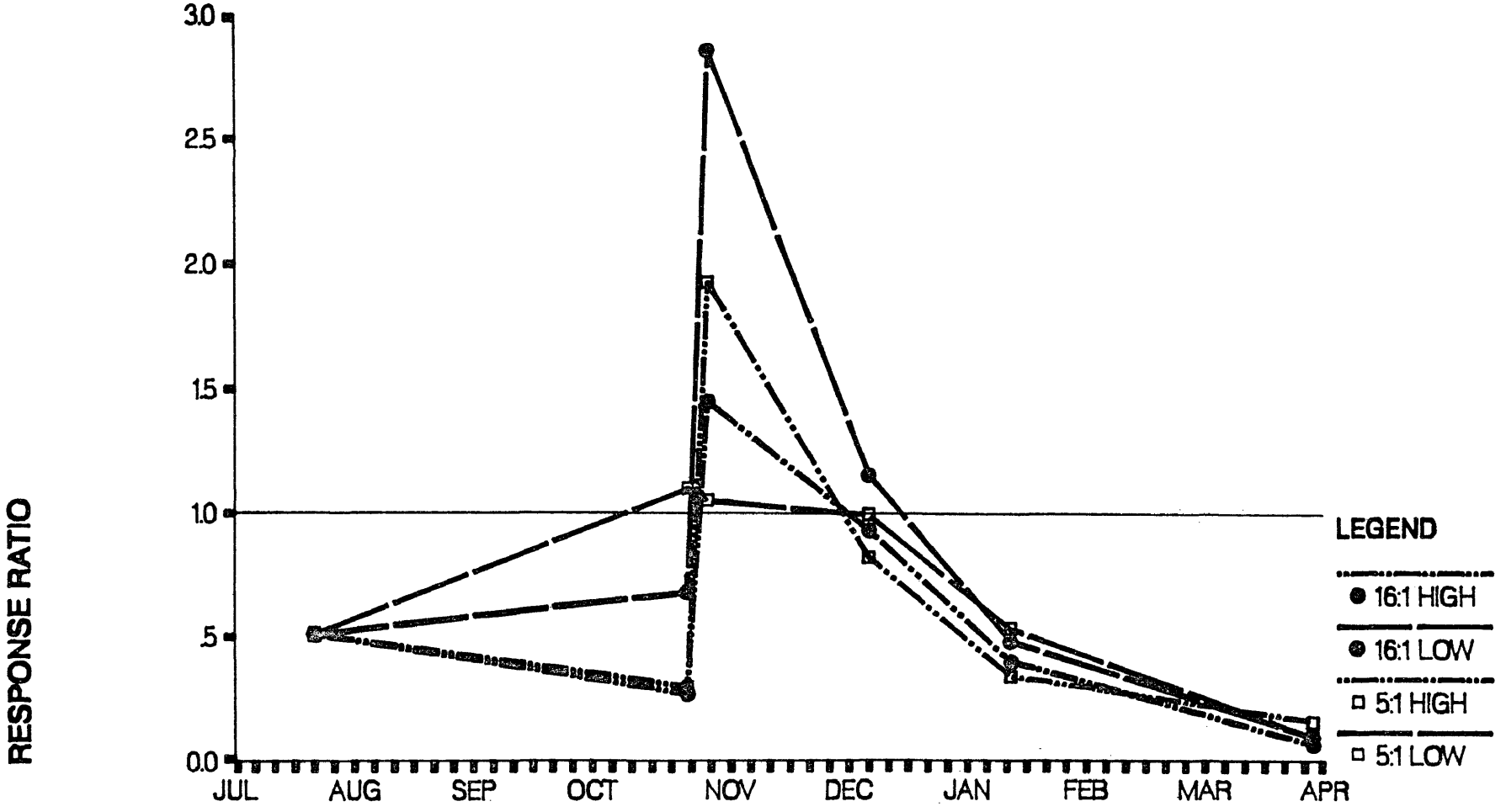

RESPONSE RATIO
3.0
2.5
2.0
1.5
1.0
.5
0.0
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
LEGEND
16:1 HIGH
16:1 LOW
5:1 HIGH
5:1 LOW

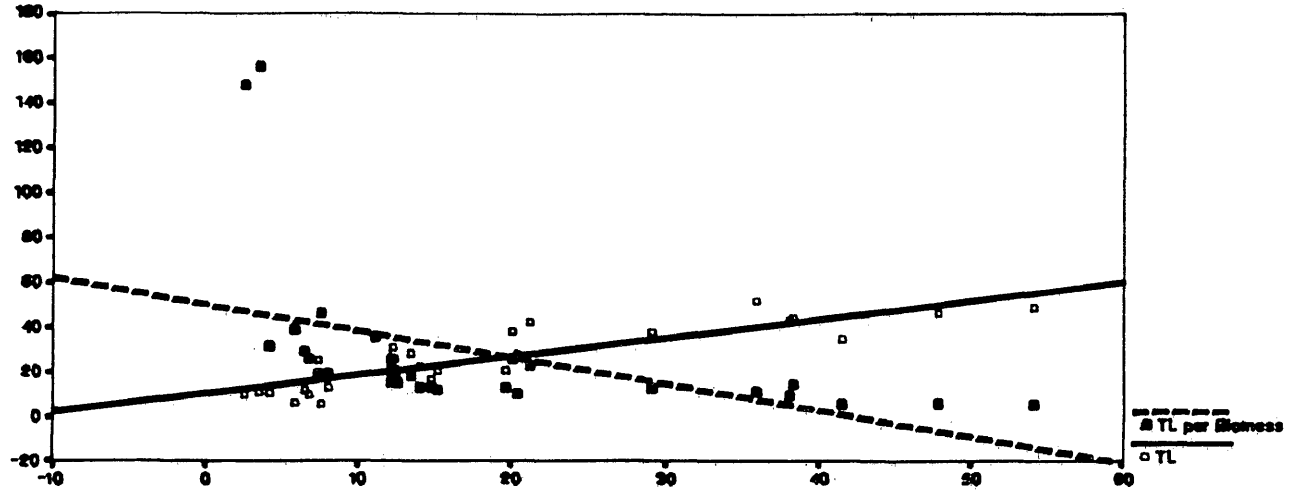
Figure 10. Contents of total lipids (TL) (mg/microcosm) and lipids per unit biomass (TL/Biomass) (ug/ug chl-a) versus intracellular PON and POP concentrations (umol).

TL-PON : $y = 0.831x + 10.5$ ($r^2 = 0.717$, $n = 30$, $P = 0.000$)

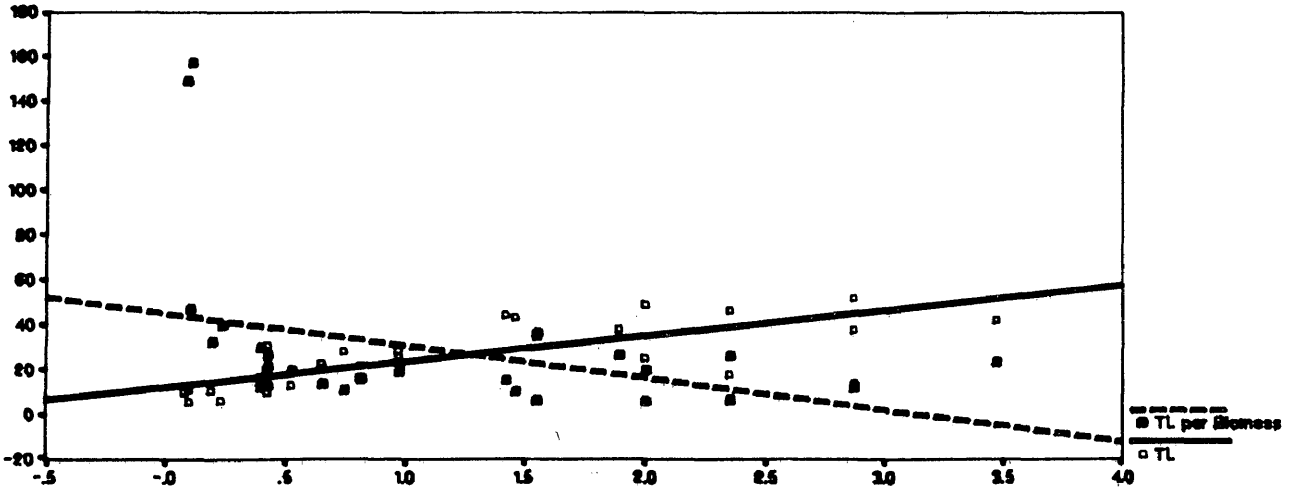
TL/Biomass-PON: $y = -1.19x + 50.1$ ($r^2 = 0.222$, $n = 30$, $P = 0.009$)

TL-POP : $y = 11.4x + 12.5$ ($r^2 = 0.616$, $n = 30$, $P = 0.000$)

TL/Biomass-POP: $y = -14.2x + 44.9$ ($r^2 = 0.146$, $n = 30$, $P = 0.037$)



PON (umol)



POP (umol)

Figure 11. Triglyceride content (as percentage of total fatty acids) versus polar lipid content (as percentage of total fatty acids).

$y = 0.585x + 39.2$ $(r^2 = 0.300, n = 30, P = 0.002)$

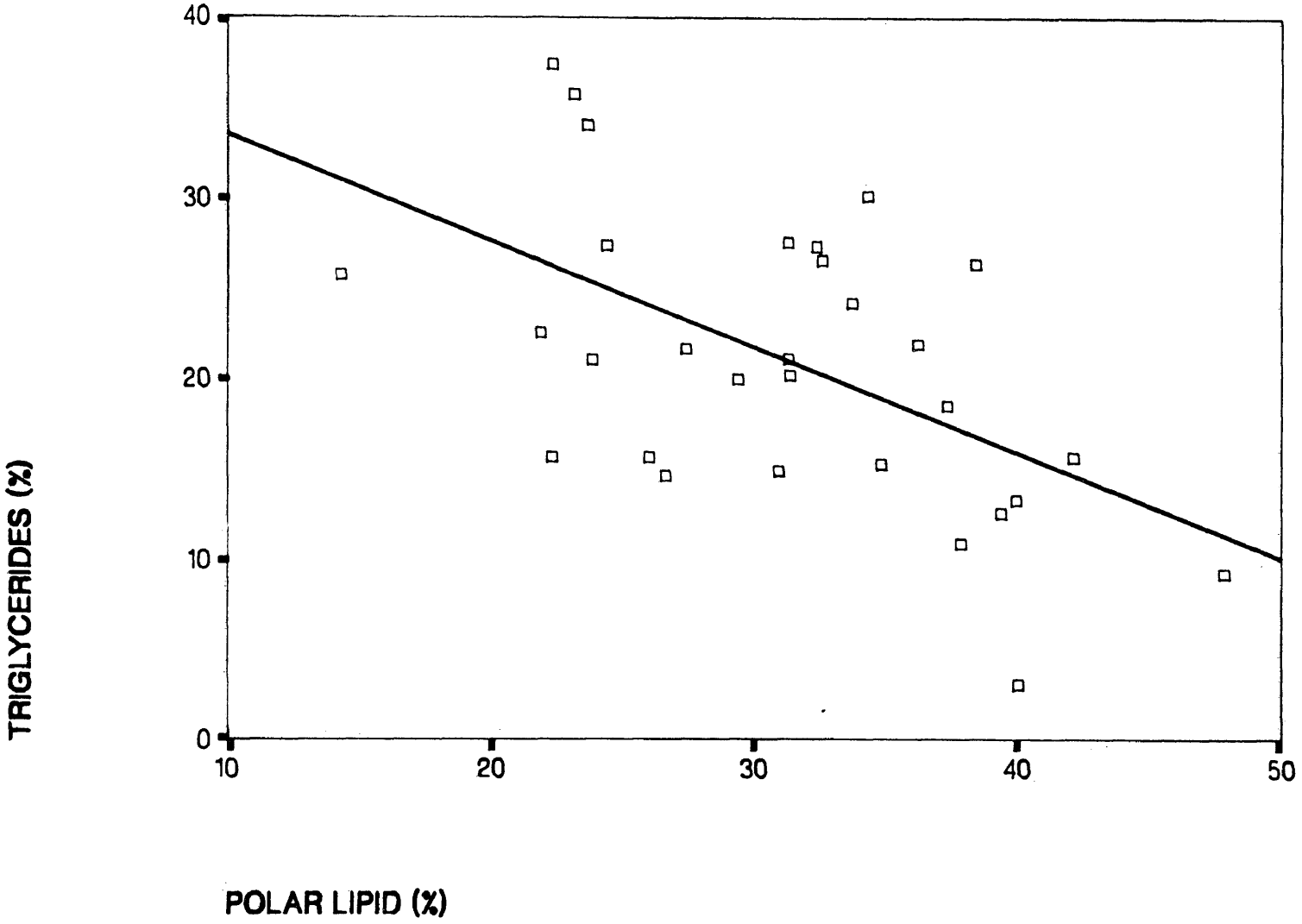

40
30
20
10
0
10
20
30
40
50
TRIGLYCERIDES (%)
POLAR LIPID (%)

Figure 12. Seasonal variations of fatty acid composition (as percentage of total fatty acids) in ambient York River microplankton during experimental period.

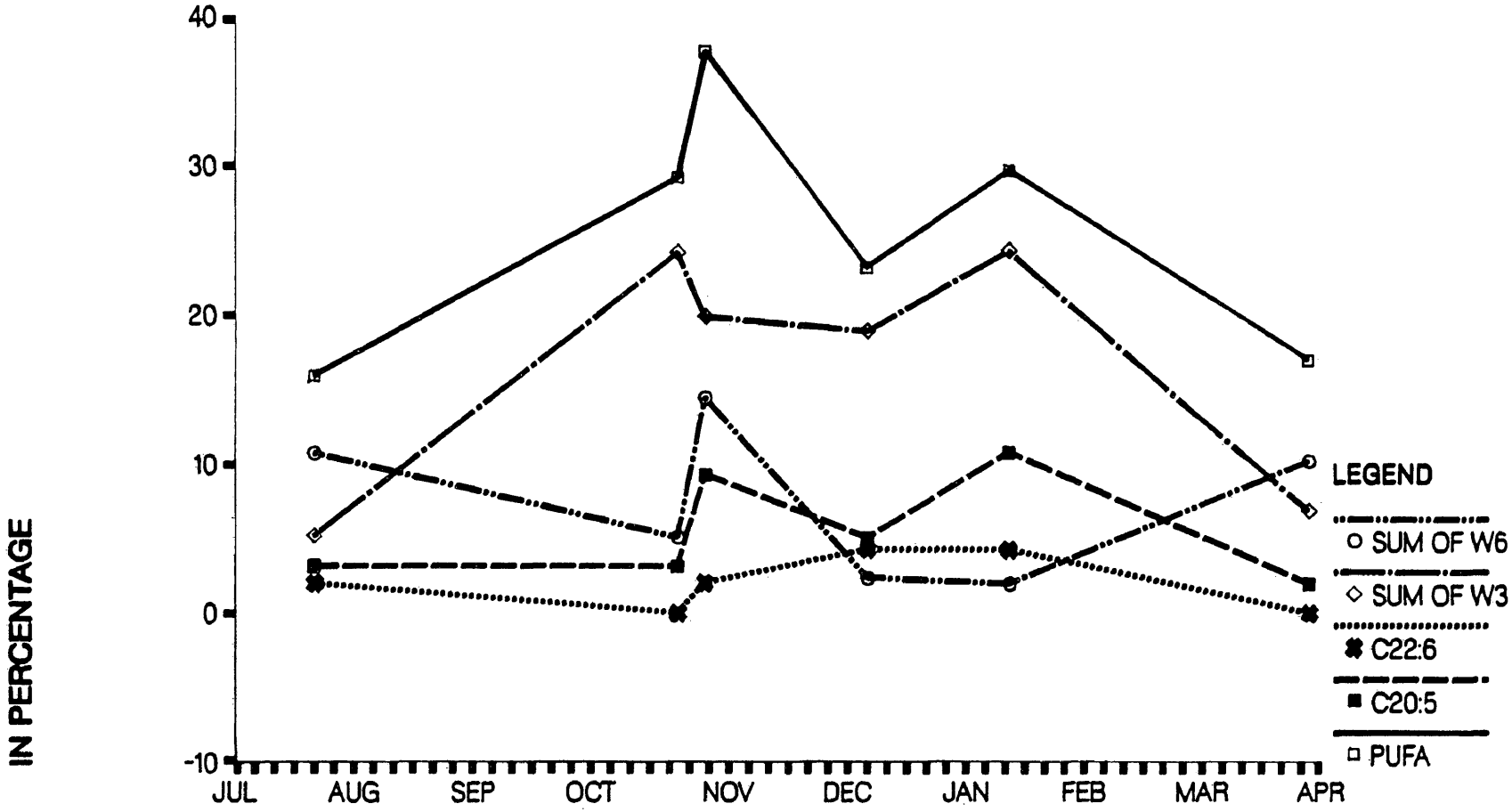
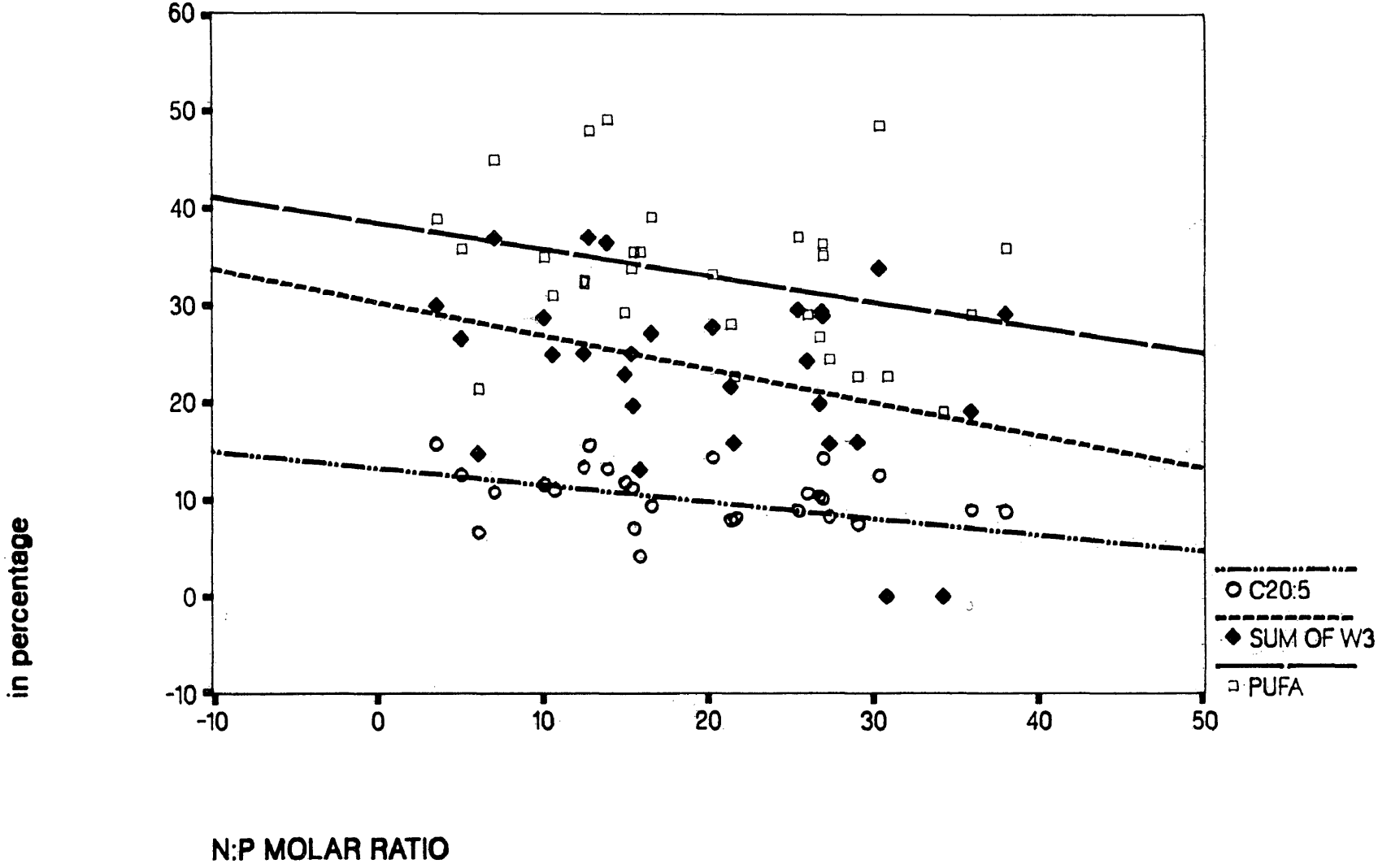

IN PERCENTAGE
40
30
20
10
0
-10
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
LEGEND
SUM OF W6
SUM OF W3
C22:6
C20:5
PUFA

Figure 13. Concentrations of 20:5w3, sum of w3 fatty acids and PUFA (as percentage of total fatty acids) versus intracellular N:P molar ratios, where x = N:P ratio and y = concentrations of fatty acids.

PUFA : $y = -0.266x + 38.4$ ($r^2 = 0.225$, $n = 30$, $P = 0.008$)

w3 fatty acids: $y = -0.342x + 30.3$ ($r^2 = 0.187$, $n = 30$, $P = 0.017$)

20:5w3 : $y = -0.171x + 13.2$ ($r^2 = 0.127$, $n = 30$, $P = 0.053$)


60
50
40
30
20
10
0
-10
-10
0
10
20
30
40
50
in percentage
N:P MOLAR RATIO
C20:5
SUM OF W3
PUFA

Appendix I
Lipid Composition of Microplankton in the Microcosms and YRW
(as percentage of total lipids)

Treatments	PL	MG	DG	ST	ALC	FFA	TG	FAE	SE	WE
7-21-88										
25:0(3)[a][b]	15.0	4.84	4.15	16.2	.62	2.13	49.4	1.98	1.96	2.10
5:1(3)	23.4	7.44	2.92	13.4	2.31	2.43	38.9	1.97	3.46	2.28
20:1(3)	23.0	7.14	3.53	13.0	1.43	3.81	39.6	1.78	4.37	2.29
C(3)	31.2	12.1	5.67	16.2	.78	4.74	21.9	<.5	3.32	4.23
YRW	50.0	8.73	<.5	6.16	1.51	3.59	22.3	<.5	2.97	4.76
10-21-88										
5:1(1)	14.3	1.49	7.54	5.49	1.69	18.7	25.8	18.9	2.14	4.05
5:1(2)	26.6	1.61	8.23	4.57	2.42	17.7	14.7	18.5	1.51	4.17
16:1(1)	23.8	2.16	8.74	7.26	1.45	16.7	21.1	11.0	2.45	5.31
16:1(2)	22.3	1.30	6.94	4.74	2.23	16.7	15.7	21.4	3.22	5.42
C(1)	21.9	4.07	10.9	9.67	<.5	13.6	22.6	8.07	3.14	6.11
C(2)	26.0	4.51	7.60	8.60	2.48	15.1	15.7	10.7	3.04	6.34
YRW	41.1	10.8	<.5	9.19	2.04	4.06	24.5	<.5	3.71	4.52
10-28-88										
5:1(1)	31.0	7.58	18.8	3.13	1.20	15.1	15.0	3.38	1.44	3.51
5:1(2)	31.4	6.96	13.8	3.59	1.49	12.7	20.3	2.89	3.47	3.46
16:1(1)	47.9	3.99	11.5	2.85	1.28	14.7	9.24	3.56	1.49	3.56
16:1(2)	34.8	5.09	11.3	3.87	2.88	16.4	15.3	2.60	3.47	4.23
C(1)	36.2	4.73	11.2	4.63	1.81	10.1	22.0	<.5	3.62	5.74
C(2)	29.4	2.62	16.1	4.71	2.06	13.2	20.2	5.25	2.47	4.11
YRW	34.4	16.1	3.37	4.71	2.54	6.62	20.2	<.5	3.48	8.53
12-9-88										
5:1(1)	31.3	22.9	3.89	3.46	1.11	7.73	21.1	<.5	3.46	5.01
5:1(2)	37.9	3.52	4.18	4.35	tr	10.5	10.9	<.5	12.9	15.8
16:1(1)	42.1	8.27	6.44	2.42	1.45	12.6	15.7	<.5	4.23	6.76
16:1(2)	40.0	5.17	9.42	4.11	2.85	12.2	13.3	.85	4.23	7.85
C(1)	32.6	12.1	5.73	2.31	1.59	7.34	26.7	.59	4.24	6.92
C(2)	38.4	2.09	4.07	3.87	2.09	6.80	26.5	1.40	4.65	10.1
YRW	42.0	7.81	4.44	3.92	4.18	5.87	21.5	<.5	4.92	5.29

cont.

Treatments	P	MG	DG	Ch	Alc	FFA	TG	FAE	ChE	Wax
1-13-89										
5:1(1)	39.4	14.2	8.49	5.02	2.01	4.93	12.6	<.5	3.77	9.54
5:1(2)	27.4	14.2	11.1	4.06	1.92	1.62	21.3	<.5	4.26	14.2
16:1(1)	41.1	16.1	17.2	2.95	1.78	2.29	3.06	.80	3.84	11.0
16:1(2)	37.3	7.57	4.80	6.80	3.60	5.24	18.6	<.5	4.75	11.4
C(1)	34.3	3.02	3.02	4.06	4.31	3.17	30.2	1.40	4.29	12.3
C(2)	33.7	3.47	2.88	5.28	3.84	2.92	24.3	1.97	7.22	14.4
YRW	41.1	12.0	3.55	5.89	5.09	3.46	20.1	<.5	2.73	6.05
3-30-89										
5:1(1)	22.4	3.40	5.22	13.8	.42	7.36	36.4	3.55	2.70	5.37
5:1(2)	23.2	2.52	6.95	12.3	.88	7.19	35.7	4.73	2.48	3.97
16:1(1)	23.7	1.56	6.65	7.63	1.25	7.55	34.0	5.71	3.14	4.85
16:1(2)	24.4	2.25	9.06	7.06	4.80	13.5	27.4	7.94	2.48	4.84
C(1)	32.4	8.89	1.57	9.47	2.61	7.45	27.4	.53	4.98	4.69
C(2)	30.5	8.53	<.5	10.8	2.65	6.75	25.5	1.81	6.66	6.88
YRW	28.7	8.70	<.5	11.3	2.99	6.21	27.0	1.46	4.70	8.97

a N:P ratios of enriched nutrients; C was control treatment.

b Dilution rates, 1: $0.25d^{-1}$; 2: $0.5d^{-1}$; 3: $0.33d^{-1}$.

Appendix II
Fatty Acid Composition of Ambient York River Microplankton
(as percentage of total fatty acids)

	7-21-88	10-21-88	10-28-88	12-9-88	1-13-89	3-30-89
14:0	9.19	10.9	8.38	6.59	8.91	15.1
15:0	<.05	<.05	<.05	1.94	<.05	<.05
16:0	43.8	30.9	24.6	35.3	31.8	43.2
18:0	13.6	13.7	7.45	13.6	8.53	5.30
SAT	65.5	55.5	40.4	57.4	49.2	63.7
16:1	10.4	12.5	12.6	10.5	14.0	12.2
18:1[a]	7.17	2.73	9.18	8.92	6.97	7.07
MONO	17.5	15.2	21.8	19.4	20.9	19.3
16:3w3	<.05	<.05	2.26	1.16	3.10	<.05
16:4w1	<.05	<.05	3.32	1.94	3.49	<.05
18:2w6	10.8	5.07	14.5	2.33	1.94	10.2
18:3w3	<.05	14.1	3.99	4.65	3.10	2.55
18:4w3	<.05	7.03	2.39	3.88	3.10	2.36
20:4w6	<.05	<.05	<.05	<.05	<.05	<.05
20:5w3	3.19	3.13	9.32	5.04	10.9	1.96
22:6w3	1.99	<.05	1.99	4.26	4.26	<.05
PUFA	15.9	29.3	37.8	23.3	29.8	17.1
w3	5.18	24.2	20.0	19.0	24.4	6.87
w6	10.8	5.07	14.5	2.33	1.94	10.2
w3/w6	.48	4.78	1.38	8.15	12.6	.67
20:5+22:6	5.18	3.13	11.3	9.30	15.1	1.96
ALK/FA[b]	1.65	1.60	2.57	.64	.65	1.67

a Sum of 18:1w7 and 18:1w9.
b Ratios between long chain hydrocarbons and fatty acids.

Appendix III
Fatty Acid Composition of Microplankton in the Microcosms
(as percentage of total fatty acids)

	7-21-88				10-21-89					
	25:0[a] (3)[b]	5:1 (3)	20:1 (3)	C (3)	5:1 (1)	5:1 (2)	16:1 (1)	16:1 (2)	C (1)	C (2)
14:0	19.5	16.7	19.7	17.0	22.4	21.8	21.9	20.5	22.0	19.6
15:0	.74	.75	<.05	<.05	1.18	1.65	1.30	1.44	1.29	1.65
16:0	30.9	37.1	34.5	42.3	16.6	16.7	18.2	15.4	17.2	20.4
18:0	3.71	4.06	3.05	6.04	2.64	1.56	2.13	2.11	6.29	4.95
SAT	54.1	57.9	57.3	65.4	42.8	41.7	43.5	39.4	46.8	46.6
16:1	30.9	19.6	24.4	16.8	22.7	23.4	27.5	23.6	24.3	27.2
18:1w7+9	2.22	7.37	3.05	4.12	2.09	1.74	2.25	1.63	4.43	3.51
MONO	33.1	26.9	27.5	20.9	24.8	25.1	29.7	25.3	28.8	30.7
16:3w3	<.05	<.05	<.05	<.05	3.18	4.40	2.72	5.45	2.29	1.86
16:4w1	<.05	<.05	<.05	<.05	2.45	2.75	2.13	3.16	1.57	1.44
18:2w6	1.98	2.26	3.29	6.87	4.91	2.66	4.73	3.06	7.15	5.36
18:3w3	<.05	<.05	<.05	<.05	3.45	3.85	2.96	4.59	2.00	2.27
18:4w3	3.09	2.55	2.11	<.05	2.00	2.20	1.89	1.72	1.86	2.68
20:4W6	5.44	5.11	5.16	3.57	<.05	<.05	<.05	<.05	<.05	<.05
20:5w3	.74	2.79	2.35	1.60	13.3	14.3	10.3	14.3	8.15	7.42
22:6w3	.74	1.80	2.35	1.60	3.09	3.03	2.01	2.97	1.43	1.65
PUFA	12.0	14.4	15.3	13.6	32.4	33.2	26.7	35.2	24.5	22.7
w3	4.57	7.06	6.81	3.20	25.0	27.8	19.9	29.0	15.7	15.9
w6	7.42	7.37	8.45	10.4	4.91	2.66	4.73	3.06	7.15	5.36
w3/w6	.62	.96	0.81	.31	5.09	10.5	4.20	9.47	2.20	2.96
20:5+22:6	1.48	4.59	4.70	3.20	16.4	17.3	12.3	17.2	9.58	9.07
ALK/FA[c]	.28	.41	.48	1.29	.20	.20	.28	.26	.41	.66

cont.

	10-28-88						12-9-88					
	5:1 (1)	5:1 (2)	16:1 (1)	16:1 (2)	C (1)	C (2)	5:1 (1)	5:1 (2)	16:1 (1)	16:1 (2)	C (1)	C (2)
14:0	20.3	15.5	17.1	17.5	16.2	18.5	7.77	4.73	6.68	10.2	9.23	9.73
15:0	1.10	1.29	1.36	1.18	.99	1.52	<.05	<.05	<.05	<.05	0.72	<.05
16:0	19.6	20.9	20.2	18.2	22.7	20.0	21.3	26.0	24.4	26.2	22.2	26.3
18:0	3.07	3.45	3.12	1.96	6.64	5.33	6.85	14.8	16.5	10.2	3.98	11.0
SAT	44.0	41.2	41.8	38.8	46.6	45.3	35.9	45.6	47.6	46.7	36.1	47.1
16:1	21.0	20.7	22.1	21.7	20.2 6	28.6	11.9	5.92	7.72	10.7	17.6	13.1
18:1w7+9	<.05	2.37	2.17	.65	4.10	3.43	3.68	13.0	9.19	3.11	10.3	11.7
MONO	21.0	23.1	24.3	22.4	24.3	32.0	15.5	18.9	16.9	13.8	27.9	24.8
16:3w3	4.93	5.82	5.29	6.54	3.81	1.90	5.01	2.07	2.92	3.56	3.17	2.95
16:4w1	4.60	5.82	5.02	7.19	3.53	1.71	5.11	2.66	3.34	5.33	4.62	4.37
18:2w6	1.64	3.45	2.99	1.70	6.50	5.14	9.51	19.8	12.5	6.67	2.12	2.08
18:3w3	7.01	5.60	6.38	3.79	4.24	1.90	9.30	5.03	5.64	6.67	5.43	2.73
18:4w3	2.96	<.05	12.2	.78	<.05	2.29	1.74	<.05	<.05	2.67	7.06	5.46
20:4w6	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20:5w3	11.5	12.5	10.7 6	15.6	8.90	8.00	12.5	4.14	7.10	9.33	8.69	7.87
22:6w3	2.30	2.59	2.31	3.27	2.12	1.71	5.42	1.78	3.97	4.88	4.89	2.62
PUFA	34.9	35.8	44.9	38.8	29.1	22.7	48.6	35.5	35.5	39.1	36.0	28.1
w3	28.7	26.5	36.9	29.9	19.1	15.8	33.9	13.0	19.6	27.1	29.2	21.6
w6	1.64	3.45	2.99	1.70	6.50	5.14	9.51	19.8	12.5	6.67	2.12	2.08
w3/w6	17.5	7.68	12.3	17.6	2.93	3.07	3.57	.68	1.57	4.06	13.8	10.4
20:5+22:6	13.8	15.1	13.0	18.8	11.0	9.71	17.9	5.92	11.1	14.2	13.6	10.5
ALK/FA	.29	.44	.32	.09	.55	.57	.53	2.17	1.11	.75	.29	.64

cont.

	1-13-89						3-30-89					
	5:1 (1)	5:1 (2)	16:1 (1)	16:1 (2)	C (1)	C (2)	5:1 (1)	5:1 (2)	16:1 (1)	16:1 (2)	C (1)	C (2)
14:0	10.2	6.67	1.18	9.78	10.0	10.6	15.8	21.5	16.2	17.0	9.07	5.09
15:0	.98	.76	1.13	<.05	.85	.97	1.58	.87	1.29	0.91	<.05	<.05
16:0	15.7	22.3	19.7	28.7	17.4	18.6	18.1	26.0	16.2	21.2	37.8	24.3
18:0	2.87	13.9	3.10	7.77	5.87	10.3	3.57	4.05	3.35	3.21	20.0	23.8
SAT	29.8	43.6	25.1	46.3	34.1	40.5	39.0	52.5	37.0	42.2	66.9	53.2
16:1	13.5	9.71	21.8	13.2	17.4	13.2	22.6	24.1	20.2	21.2	3.00	1.99
18:1w7+9	4.64	7.43	3.98	6.75	11.4	20.4	7.31	2.12	6.39	5.33	11.6	22.1
MONO	18.2	17.1	25.8	19.9	28.4	33.5	29.9	26.2	26.6	26.5	14.6	24.1
16:3w3	5.69	3.24	7.51	2.70	3.98	3.05	1.81	1.35	3.03	2.79	<.05	<.05
16:4w1	8.70	6.19	10.4	6.42	4.56	4.39	2.93	2.12	4.04	2.97	<.05	<.05
18:2w6	2.22	10.2	2.27	2.36	2.90	1.93	3.21	4.53	2.94	1.82	19.0	22.7
18:3w3	3.73	5.62	2.71	3.72	1.39	1.41	1.12	4.43	3.63	.97	<.05	<.05
18:4w3	7.65	<.05	9.21	4.39	8.88	4.83	8.34	1.25	8.85	7.75	<.05	<.05
20:4w6	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20:5w3	15.5	11.7	13.1	11.2	8.89	7.81	11.1	6.65	10.1	10.6	<.05	<.05
22:6w3	4.45	2.29	3.97	3.04	6.49	2.60	2.61	1.06	3.84	2.18	<.05	<.05
PUFA	47.9	29.2	49.1	33.8	37.1	15.6	31.0	21.3	36.4	29.1	19.0	22.7
w3	37.0	22.9	36.5	25.0	29.6	9.29	24.9	14.7	29.5	24.3	<.05	<.05
w6	2.22	10.2	2.27	2.36	2.90	1.93	3.21	4.53	2.94	1.82	19.0	22.7
w3/w6	16.7	2.24	16.1	10.6	10.2	4.81	7.75	3.23	10.0	13.4	<.05	<.05
20:5+22:6	20.0	14.0	17.1	14.2	15.4	10.4	13.7	7.62	13.9	12.8	<.05	<.05
ALK/FA	.18	1.44	.18	.32	.49	.75	.27	.43	631	.26	3.73	2.48

a N:P ratios of enriched nutrients, C was control treatment.

b Dilution rates, 1: $0.25d^{-1}$; 2: $0.5d^{-1}$; 3: $0.33d^{-1}$.

c Ratios between long chain alkenes and fatty acids.

Appendix IV
MANOVA Results
Effect of Time, Dilution Rate and Nutrient Treatment

BIOMASS AND LIPID CONTENT

		POC	CHL BIO	TL	LIP/CHL	TL/C
DIL	F[a]	1.09	0.17	1.10	0.05	0.28
	P[b]	0.31	0.68	0.31	0.83	0.60
TIME	F	11.79	10.20	12.94	2.84	4.80
	P	0.00	0.00	0.00	0.05	0.00
TRT	F	7.02	5.51	9.77	3.79	0.26
	P	0.00	0.01	0.00	0.04	0.77

LIPID COMPOSITION

		P	MO	DI	ST	ALC	FFA	TG	FAE	SE	WE
DIL	F	1.25	3.54	0.25	0.01	5.61	0.50	0.08	2.26	3.26	5.48
	P	0.31	0.07	0.62	0.94	0.25	0.48	0.77	0.15	0.09	0.03
TIME	F	14.09	2.84	5.87	12.14	9.78	45.02	6.28	22.11	4.46	16.68
	P	0.00	0.05	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00
TRT	F	2.70	1.48	2.68	1.64	18.00	14.27	4.80	2.66	1.20	1.67
	P	0.09	0.25	0.09	0.22	0.16	0.00	0.02	0.09	0.32	0.21

FATTY ACID COMPOSITION

		SAT	MONO	PUFA	W3	W6	20:5	22:6	W3/W6	AL/FA
DIL	F	2.23	0.07	8.88	4.74	0.08	0.37	4.50	0.66	0.63
	P	0.15	0.79	0.00	0.04	0.77	0.55	0.05	0.42	0.44
TIME	F	2.49	2.16	3.92	2.52	1.46	4.62	4.69	1.29	1.81
	P	0.07	0.11	0.02	0.07	0.25	0.00	0.00	0.30	0.16
TRT	F	2.05	3.05	11.58	8.61	0.94	10.99	1.55	2.18	2.58
	P	0.15	0.07	0.00	0.00	0.41	0.00	0.24	0.14	0.10

a F scores.
b Possibility or significance.

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